OPTIMIZING GROWTH FOR AQUACULTURE OF JUVENILE BLACK SEA BASS *CENTROPRISTIS STRIATA* L. : EFFECTS OF TEMPERATURE, SALINITY, COMMERCIAL DIET AND FEEDING RATION.

by

CHARLES F. COTTON

(Under the Direction of Dr. Randal L. Walker)

ABSTRACT

A new market has emerged for live black sea bass, *Centropristis striata* L., in fish markets of the northeast United States and Canada. Efforts to culture black sea bass have been hampered by the lack of information regarding optimal grow-out conditions. This research was designed to determine optimal commercial diet, feeding ration, temperature, and salinity for growth of hatchery-reared, juvenile black sea bass. Optimal diet was Zeigler Salmon Starter (compared to Nelson and Son's Silver Cup Salmon Crumbles, Trout Crumbles, and Rangen Trout and Salmon Starter). Optimal daily feeding ration was 5% (compared to 2.5% and 7.5%). Optimal water temperature was 25º C (compared to 15º C, 20º C, and 30º C), and optimal salinity was 20 ppt or 30 ppt (compared to 10 ppt). Additionally, growth rates, feed conversion ratio, and mortality were calculated in each experiment. This information will be a valuable guide for culturing juvenile black sea bass.

INDEX WORDS: Centropristis striata, Aquaculture, Diet, Ration, Temperature, Salinity, Growth

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Black sea bass *Centropristis striata striata* (Linnaeus, 1758) are found along the Atlantic Coast of the United States from Massachusetts to Florida as well as the Gulf of Mexico. This species provides an important recreational and commercial fishery throughout its range (Musick and Mercer 1977). North of Cape Hatteras, fish undergo seasonal migrations, generally inshore and northward in the spring and offshore and southward in the fall, and those south of the Cape exhibit only localized movements (Cupka et al. 1973, Mercer 1989). This, in part, has led to the assumption that two populations exist along the Atlantic coast, separated at Cape Hatteras (Mercer 1989). Furthermore, Shepherd (1991) asserted the possibility of more than one stock north of Cape Hatteras. Black sea bass *C. s. melana* in the Gulf of Mexico become resident once established on a reef (Hood et al. 1994).

The genus *Centropristis* was proposed in 1829 by Cuvier and Valenciennes in Histoire Naturelle des Poissons (Weed 1937). Ginsberg (1952) reported that a separate species (*C. melana*) exists in the Gulf of Mexico. However, Miller (1959) later proposed a subspecific designation (*C. s. melana*), based on morphometric and meristic data from a larger sample size than that which Ginsberg used.

Black sea bass are in the family Serranidae and are protogynous hermaphrodites (Lavenda 1949). Most fish function first as females and later transform into males. Significant differences have been found in sex ratio according to size and age (Lavenda

1949; Wenner et al. 1986). In the Wenner et al. (1986) study, male to female ratio was 1: 8.50 in the smallest size group, and the ratio was 1: 0.20 for the largest size group. Other sexually dimorphic traits reported by Lavenda (1949) include an adipose hump in reproductively active males, different head shape and appearance, differences in body shape and fin structures, and a more vivid coloration in males, which is pronounced during spawning season.

In the South Atlantic Bight, black sea bass spawn from January to June with a peak spawn from March to May (Wenner et al. 1986). A minor spawn also occurs during September and October in the South Atlantic Bight (Wenner et al. 1986). North of Cape Hatteras, spawning occurs from June to October (Kendall 1972). Embryology has been reported by Wilson (1891) and larvae were described by Kendall (1972).

Black sea bass are primarily associated with live-bottom reefs, sponges, wrecks and other similar structures. Kendall (1972) reported that juveniles become demersal at approximately 13-24 mm total length (TL). Juveniles inhabit both estuarine and offshore reef areas (Cupka et al. 1973; Musick and Mercer 1977). Adult black sea bass are commonly sought by both commercial and recreational anglers. In Georgia, black sea bass are possibly the most conspicuous predators of the live-bottom reefs, such as Gray's Reef National Marine Sanctuary (Gilligan 1989). Low and Waltz (1991) list black sea bass as the most important bottom fish of the artificial reefs and hard bottom areas of South Carolina. They are classified as an opportunistic carnivore and consume the following prey items in order of importance: crustaceans, fish, mollusks, and echinoderms (Cupka et al. 1973).

Congeners often confused with *C. striata* include *C. ocyurus*, the bank sea bass and *C. philadelphica*, the rock sea bass. Neither of these species reaches a marketable size and therefore is not considered to be commercially valuable.

Labor-intensive pot traps and baited wire traps are the principle gear for the sea bass fishery (Rivers 1966; Frame and Pearce 1973; Low 1982; Vaughan et al. 1995). Other fishers use hook and line, gill nets, haul seines, pound nets, and trawls (Vaughan et al. 1995). A commercial fishery exists but recreational anglers harvest more black sea bass annually. For the period of 1979-1990, recreational fishing accounted for 43% (by weight) of landings, whereas commercial landings accounted for 33% and headboat landings accounted for 24% of the total harvest of black sea bass (Vaughan et al. 1995). Berlinsky et al. (2000) report that in the northeastern United States, demand typically exceeds supply for black sea bass. Musick and Mercer (1977) state that black sea bass appear to be overharvested and Vaughan et al. (1995) report a declining catch per unit effort (CPUE) in the southeastern United States.

Regulation of black sea bass in Georgia is covered under the Fishery Management Plan for the Snapper Grouper Fishery of the South Atlantic Region (SAFMC 1983). This management plan implemented a 20.3-cm (8-inch) (TL) minimum size limit for black sea bass. In February 1999, Amendment 9 to the Fishery Management Plan for Black Sea Bass (SAFMC 1998) increased the minimum size limit to 25.4-cm (10 inches) (TL) in the South Atlantic region. Charter boat captains complain about the new size limit, noting that black sea bass was once a very reliable catch for their customers. When an offshore chartered fishing trip ended unsuccessfully, captains could always stop at the nearshore reefs and catch numerous black sea bass so their customers would be able to take fish home (Gary Kinard, personal communication). Now with the 25.4-cm size limit, these captains find that catching substantial numbers of legal-size black sea bass is difficult.

A small commercial demand exists for black sea bass in Georgia's fish markets with wholesale prices ranging from \$3.85 to \$9.90 per kilogram and averaging \$5.50 per kilogram for whole fish (Charlie Phillips, personal communication). Whole fish are preferred by the seafood brokers because of the poor yield of fillets (Charlie Phillips, personal communication). Cupka et al. (1973) report a yield of approximately 39% of initial body weight for black sea bass fillets. Live black sea bass have been sold in fish markets of the northeast United States and Canada with wholesale prices ranging from \$11.00 to \$17.60 per kilogram (Gary Kinard, personal communication). These premium prices for live fish are being offered primarily by the sushi industry, which depends on an extremely fresh product. These sushi brokers prefer a shipment of uniform sized fish of approximately 900 grams (2 lbs.).

Seeking the premium market value of live fish, a resourceful fisherman in McIntosh County, Georgia recently began culturing pot-trapped, minimum-legal-size black sea bass and rearing them in land based tanks to a 900-gram size for the live market. He approached the University of Georgia Marine Extension Service for consultation and research was undertaken to develop methods for culturing black sea bass. Aquaculturists and scientists immediately realized that the industry could not rely on trapping wild fish as a sustainable source. While this practice promises substantial economic benefits for the fisherman, it adds more pressure to a heavily fished population and is therefore not a sustainable practice. In addition, the previously mentioned regulations make catching legal-size fish for market rearing more difficult. To satisfy market demand and support an emerging industry while reducing fishing pressure, the aquaculture industry should utilize hatchery reared black sea bass fingerlings, rather than trapping minimum-legal-size wild fish. Utilizing hatchery-reared fish can help to protect native stocks, while providing commercial fishers an alternate form of income. Therefore, the Marine Extension Service contracted a commercial hatchery (Southland Fisheries Corporation, Edisto, SC) to spawn black sea bass. The hatchery produces fingerling-size fish for experimentation and ultimately for the industry to grow out and sell to sushi markets. The goal of this process is to provide an economically viable and sustainable method for culturing hatchery reared fingerling black sea bass to a market size of approximately 1-kg.

Black sea bass have great potential for mariculture; they are hardy, grow rapidly and have high market value (Hoff 1970; Roberts et al. 1976; Harpster et al. 1977; Tucker 1984; Kim 1987; Costa and Provenzano 1993; Berlinsky et al. 2000; Kupper et al. 2000; Walker and Moroney 2000). Previous research on black sea bass mariculture focused primarily on brood stock management and spawning techniques (Hoff 1970; Roberts et al. 1976; Tucker 1984; Chappell et al. 2001; Stuart et al. 2001), larval development and culture (Hoff 1970; Roberts et al. 1976; Tucker 1984; Berlinsky et al. 2000), grow-out of juveniles, (Harpster et al. 1977; Kim 1987; Berlinsky et al. 2000) and grow-out of sub-adults and adults (Kim 1987; Costa and Provenzano 1993; Berlinsky et al. 2000; Kupper et al. 2000; Walker and Moroney 2000; Copeland et al. 2001). The methodology of spawning and hatchery care is well documented, but research is lacking in the growth of hatchery-reared fingerlings to market size of 900 grams. The conclusions of this body of literature devoted to black sea bass aquaculture indicate that black sea bass is an excellent candidate for aquaculture.

The research presented in this thesis includes determinations of optimal feeding ration, commercial diet, temperature, and salinity for growth of juvenile black sea bass. This research is being conducted as part of an effort to determine the feasibility of growing hatchery reared fingerlings to a desirable market size of approximately 1-kg. The long-term goal is to provide a method that is economically and energetically advantageous to the current method of trapping minimum-legal-size wild fish and raising them to a marketable size.

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CHAPTER 2

OPTIMAL COMMERCIAL DIET AND FEEDING RATION FOR BLACK SEA BASS *CENTROPRISTIS STRIATA* FINGERLINGS[1]

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Abstract

A new market for live black sea bass, *Centropristis striata* L., is emerging in the fish markets of the northeast United States and Canada. Aquaculturists will need to know optimal commercial diet and feeding ration for growing black sea bass to keep operating costs at a minimum and insure maximum profit. In this study, optimal commercial diet and feeding ration were experimentally determined for juvenile black sea bass (0.8 – 1.3 grams). The diet experiment compared growth rates for fish fed four commercial diets, Rangen Trout and Salmon Starter (R), Nelson and Son's Silver Cup Trout Crumbles (SCT), Nelson and Son's Silver Cup Salmon Crumbles (SCS), and Zeigler Salmon Starter (Z). The ration experiment compared growth rates for fish fed at 2.5%, 5.0%, and 7.5% rations (dry weight of food/ wet weight of fish). At the end of the diet experiment (18 weeks), Analysis of Variance (ANOVA) test found significant differences ($p < 0.0001$) in weight among treatments. Tukey's studentized range test ($\propto = 0.05$) showed that fish fed diet Z (14.5 ± 0.92 g) were significantly larger than fish fed diet R (11.4 ± 0.93 g). Diets Z and R both produced significantly larger fish than diets SCS (7.3 ± 0.85 g) and SCT (4.5 ± 0.29 g). At the end of the feeding ration experiment (10 weeks), ANOVA found significant differences ($p = 0.0475$) in weight among treatments. Duncan's test ($\propto = 0.05$) showed that fish fed a 7.5% (4.61 ± 0.24 g) ration and a 5.0% (4.49 ± 0.24 g) ration were not significantly different in weight. Both of these treatments produced significantly larger fish than a feeding ration of 2.5% (3.59 ± 0.21 g).

Keywords: Centropristis striata, aquaculture, diet, ration, growth.

Introduction

Black sea bass, *Centropristis striata* L., are commonly found along the eastern seaboard from Cape Cod, Massachusetts to Cape Canaveral, Florida (Musick and Mercer 1977). Like many other members of the family Serranidae (sea basses and groupers), black sea bass are protogynous hermaphrodites and sexually dimorphic (Lavenda 1949). A commercial fishery exists but recreational anglers harvest more black sea bass annually (Vaughan et al. 1995).

A new market for live black sea bass is emerging to satisfy the demand for sushi grade fish in the ethnic fish markets of the northeast United States and Canada. Berlinsky et al. (2000) state that the demand for black sea bass in the northeast United States usually exceeds supply. In Georgia's fish markets, black sea bass wholesales for approximately $5.50 per kilogram for whole fish (Charlie Phillips, personal communication). Live black sea bass, however, can be transported to markets in the northeast United States and sold for $11.00-$17.60 per kilogram (Gary Kinard, personal communication). In Georgia and a few other states on the Atlantic Coast, the live market has been supplied by pot-trapped fish taken from nearshore reefs. Fishers trap minimum-legal-sized fish and transport them to land-based tanks. Here the fish are grown to a market size of approximately 900–g and hauled alive to the markets of the northeast United States.

Catch per unit effort (CPUE) for black sea bass has declined in the South Atlantic Bight (Vaughan et al. 1995) presumably because of current levels of fishing pressure, both commercial and recreational. In February 1999, the minimum size limit for black sea bass was increased from 20.3-cm (8-in) to 25.4-cm (10-in), TL (SAFMC 1998). This new size regulation has made the practice of trapping black sea bass more difficult as fishers find it harder to catch great numbers of minimum-legal sized fish. To support this emerging aquacultural industry while protecting wild stocks of black sea bass, mariculture of this species should rely on fish hatcheries as a source of fingerlings rather than trapping wild adults and sub-adults.

Although spawning techniques have been well documented, commercial culture of this species has yet to develop. Previous research on black sea bass mariculture includes studies of brood stock management and spawning techniques (Hoff 1970; Roberts et al. 1976; Tucker 1984; Chappell et al. 2001; Stuart et al. 2001), larval development and culture (Hoff 1970; Roberts et al. 1976; Tucker 1984; Berlinsky et al. 2000), grow-out of juveniles, (Harpster et al. 1977; Kim 1987; Berlinsky et al. 2000) and grow-out of sub-adults and adults (Kim 1987; Costa and Provenzano 1993; Berlinsky et al. 2000; Kupper et al. 2000; Walker and Moroney 2000; Copeland et al. 2001). Previous research involving grow-out of juvenile black sea bass is of particular interest in this study. Harpster et al. (1977) compared commercial and natural diets for juvenile black sea bass. Kim (1987) examined various feeding regimes with energy budget calculations, comparisons of commercial and natural diets, and a theoretical calculation of feeding ration based on satiation feeding. Berlinsky et al. (2000) investigated factors associated with larviculture, commercial diets of larvae, juveniles, and sub-adults, as well as the effect of salinity on growth of juvenile black sea bass.

This work was intended to build upon the studies of grow-out of juvenile black sea bass by experimentally determining the optimal feeding ration and commercial diet for this species. This research should serve as a starting point upon which to build an understanding of the diet and feeding ration of this fish and how environmental and physiological factors affect these important growth parameters.

Materials and Methods

Brood stock were captured by pot traps off the coast of Georgia and later subjected to hormone induced spawning to produce the fingerlings used in the diet experiment. These fingerlings (mean weight = 0.55 g, SE = ± 0.02 g) were purchased in December 1998 from Southland Fisheries Corporation in Edisto, SC. Fish were kept in 600-L fiberglass holding tanks with a flow-through circulation of estuarine water from the Skidaway River, and supplemental aeration was provided by airstones. Fish were fed INVE larval fish meal (size #1 crumble) during this holding period.

Brood stock were captured by pot traps off the coast of Virginia and later subjected to hormone induced spawning to produce the fingerlings used in the feeding ration experiment. This second batch of fingerlings (1.29 ± 0.02 g) was purchased from Southland Fisheries Corporation in September 2000 and immediately used to stock the feeding ration experiment.

Diet Experiment

Fish (0.82 ± 0.02 g) were raised for 122 days in 65-L fiberglass tanks with a flow-through circulation of estuarine water from the Skidaway River. The flow rate for each tank was maintained at approximately 1.8 L min^{-1} (approximately 40 exchanges day^{-1}). Tanks were kept under constant light (40 lx) and provided with supplemental aeration by airstones. Fish were fed one of four commercial fingerling feeds (size #3 crumble) at a

5% daily ration (dry weight of food / wet weight of fish) by using the total biomass of fish in each tank to calculate feeding ration. The feeds used were Rangen Trout and Salmon Starter (R), Nelson and Son's Silver Cup Trout Crumbles (SCT), Nelson and Son's Silver Cup Salmon Crumbles (SCS), and Zeigler Salmon Starter (Z). Nutritional composition and cost (at the time of this experiment) of each feed are listed in Table 2.1. Twelve tanks were stocked with 50 fish per tank. A random number table was used to assign treatments (n=4) and replicates (n=3) to each tank after stocking.

Fish were weighed and tanks were drained and cleaned biweekly in the following manner. A subset of 30 fish, randomly chosen from each tank, was individually weighed to obtain mean weight and standard error for each tank. Afterwards, all fish in the tank were simultaneously weighed to obtain total biomass in the tank for computation of feeding ration. Fish were weighed in a tared, 1-L beaker of seawater on an Acculab V-1200 balance. Fish were not anesthetized during weighing. During tank cleaning, flow of incoming seawater was adjusted to 1.8 L min^{-1} if necessary to ensure equal flushing of all tanks.

Treatment means (weight) were analyzed by Analysis of Variance (ANOVA) and Tukey's studentized range test with The SAS System for Windows v6.12 software. Mortality data (expressed in decimal form) were arcsine transformed for each replicate. The transformed data were analyzed by ANOVA using Microsoft Excel 2000 software.

Table 2.1 - Nutritional information for the four commercial diets used in this experiment. The feeds used were Nelson and Son's Silver Cup Trout Crumbles (SCT), Nelson and Son's Silver Cup Salmon Crumbles (SCS), Zeigler Salmon Starter (Z), and Rangen Trout and Salmon Starter (R).

	Nutritional composition					
Commercial feed	% Protein	% Lipid	% Carbohydrate	% Fiber	Digestible energy (kcal/g)	Cost per kg.
SCT	45	11	18	3	3.85	$1.30
SCS	48	11	15	3	3.95	$1.32
Z	50	15	15	2	4.25	$1.39
R	50	16	8	3	4.13	$1.23

Relative growth rate (RGR), specific growth rate (SGR), and daily weight gain (DWG), were calculated using Formulas 2.1-2.3, respectively.

(2.1) RGR = 100 X (final weight – initial weight)/(initial weight)

(2.2) SGR = 100 X [ln (final weight) – ln (initial weight)]/(time in days)

(2.3) DWG = (final weight – initial weight)/(time in days)

Feed conversion ratio was calculated using Formula 2.4. To account for weight loss attributed to mortality, an adjusted FCR was calculated using Formula 2.5.

(2.4) FCR = (weight of feed consumed)/(final total biomass – initial total biomass)

(2.5) FCR_{adj} = (weight of feed consumed)/[(final total biomass) – (number of fish remaining at the end of the experiment) X (initial mean weight)].

Water temperature, salinity, dissolved oxygen, ammonia concentration, and pH were measured weekly. Dissolved oxygen was measured with an Orion (model 830) dissolved oxygen meter, and pH was measured with an Oakton (model pH Testr2) pH meter. Salinity and temperature were measured daily with a refractometer and alcohol thermometer in the Skidaway River, adjacent to the intake for the lab's flow-through seawater system. Total ammonia nitrogen (TAN) was measured with a LaMotte colorimeter (model SMART), which uses an adaptation of the Nesslerization method.

Feeding Ration Experiment

Fish (1.29 ± 0.02 g) were raised for 70 days in 65-L fiberglass tanks with a flow-through circulation of estuarine water from the Skidaway River. The flow rate for each tank was maintained at approximately 1.8 L min^{-1} (approximately 40 exchanges day^{-1}). Tanks were kept under constant light (40 lx) and provided with supplemental aeration by airstones. Fish were fed Zeigler Salmon Starter (size #3 crumble) at three daily feeding rations (dry weight of food/ wet weight of fish). Nutritional composition of this feed is listed in Table 2.1. The daily feeding rations tested were 2.5%, 5.0%, and 7.5%, and were calculated using the total biomass of fish in each tank. Nine tanks were stocked with 75 fish per tank. A random number table was used to assign treatments (n=3) and replicates (n=3) to each tank after stocking. Every 2 weeks, tanks were cleaned and new rations were calculated and adjusted by measuring the total biomass in each tank. Additionally, on weeks 2, 6 and 10, a subset of 30 fish from each tank was individually weighed to compute mean weight and standard error for each tank. Fish weighing and tank maintenance was performed as in the diet experiment. Treatment means (weight), mortality data and feeding ration were calculated and analyzed as in the diet experiment. Growth rates (RGR, SGR, and DWG) and feed conversion ratio (FCR and FCR_{adj}) were calculated as in the diet experiment.

Water temperature, salinity, dissolved oxygen, ammonia concentration, and pH were measured weekly in the same manner as the diet experiment.

Results

Diet Experiment

In the diet experiment (Fig. 2.1), significant differences ($p < 0.0001$) in weight of fish were found after 10 weeks, with diets Z and R producing larger fish than diets SCS and SCT. By week 18, Tukey's studentized range test ($\propto = 0.05$) showed that fish fed diet Z (14.5 ± 0.92 g) were significantly larger than fish fed diet R (11.4 ± 0.93 g). Diets Z and R both produced significantly larger fish than diets SCS (6.9 ± 0.85 g) and SCT (4.5 ± 0.29 g). The simultaneous multiple comparison of all treatments showed that the two Silver Cup diets did not produce significantly different sized fish. However, a single pairwise comparison ($\propto = 0.05$) of SCS and SCT using Tukey's test showed that SCS produced significantly larger fish than SCT.

Growth and feed conversion data are presented in Table 2.2. Weight gain, RGR, SGR, and DWG were highest for fish fed diet Z. Similarly, feed conversion ratio was lowest for diet Z.

Mortality for each treatment is as follows, 64.7% ± 25.2% for diet Z, 64.7% ± 24.3% for diet R, 85.3% ± 7.7% for diet SCS, and 61.3% ± 6.3% for diet SCT. There were no significant differences ($p = 0.7879$) in mortality among treatments.

Water quality data for this experiment are presented in Figures 2.2 – 2.4. Temperature ranged from 12.1° C – 26.6° C, salinity ranged from 23 – 32 ppt, dissolved oxygen ranged from 5.36 – 6.93 ppm and TAN ranged from 0.00 – 0.89 ppm throughout the experiment. No graph was generated for pH because the value was virtually constant (≈ 7.5 – 7.8) throughout the experiment.

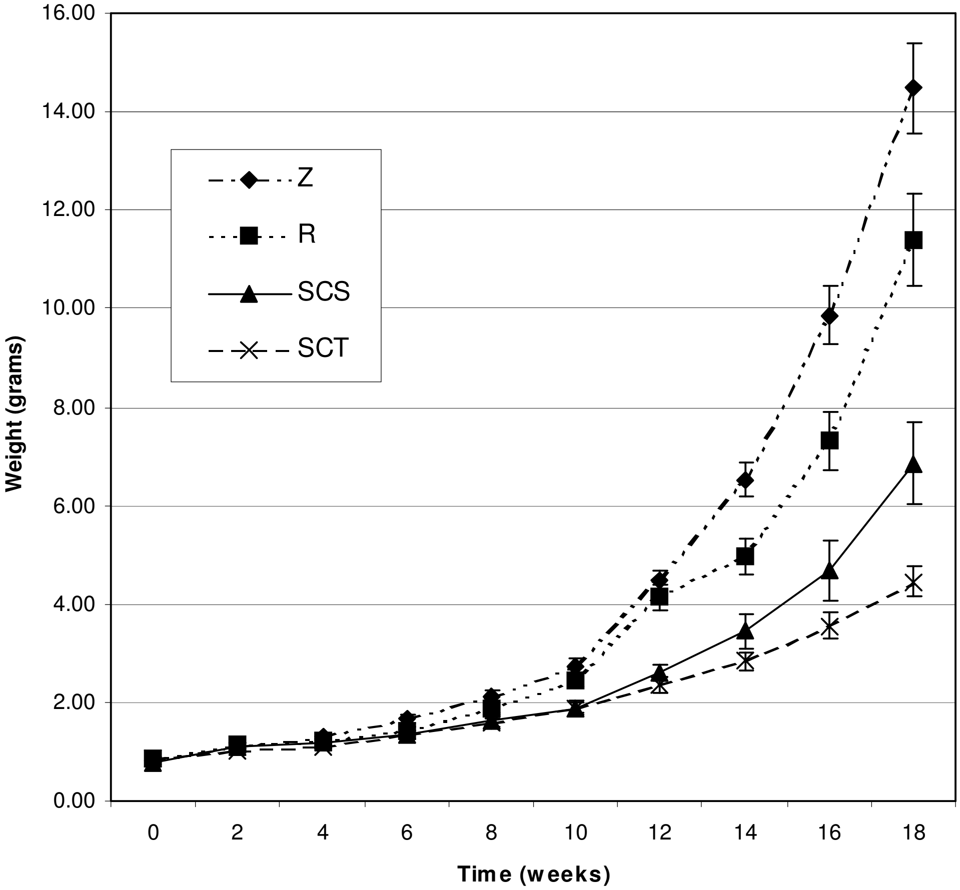

Figure 2.1 – Mean weight (± SE) versus time for juvenile black sea bass fed four commercial diets, Z = Zeigler Salmon Starter, R = Rangen Trout and Salmon Starter, SCS = Nelson's Silver Cup Salmon Crumbles, and SCT = Nelson's Silver Cup Trout Crumbles. Standard errors were very small until week 12 and therefore error bars are indistinguishable for times prior to week 12.

Table 2.2 – Initial and final weights, weight gain, relative growth rate (RGR), specific growth rate (SGR), daily weight gain (DWG), feed conversion ratio (FCR), and adjusted feed conversion ratio (FCR_{adj}) for juvenile black sea bass fed the four commercial diets used in this experiment. The feeds used were Nelson and Son's Silver Cup Trout Crumbles (SCT), Nelson and Son's Silver Cup Salmon Crumbles (SCS), Zeigler Salmon Starter (Z), and Rangen Trout and Salmon Starter (R).

	Variable							
Commercial feed	Initial weight (g)	Final weight (g)	Weight gain (g)	RGR (%)	SGR (%/day)	DWG (g/day)	FCR	FCR_{adj}
Z	0.80 ± 0.05	14.49 ± 0.92	13.69	1711.3	2.37	0.112	1.84	1.66
R	0.84 ± 0.04	11.39 ± 0.93	10.55	1256.0	2.13	0.086	2.39	2.09
SCS	0.79 ± 0.06	6.86 ± 0.85	6.07	768.4	1.78	0.050	16.41	4.79
SCT	0.86 ± 0.05	4.46 ± 0.29	3.60	418.6	1.35	0.030	5.02	3.54

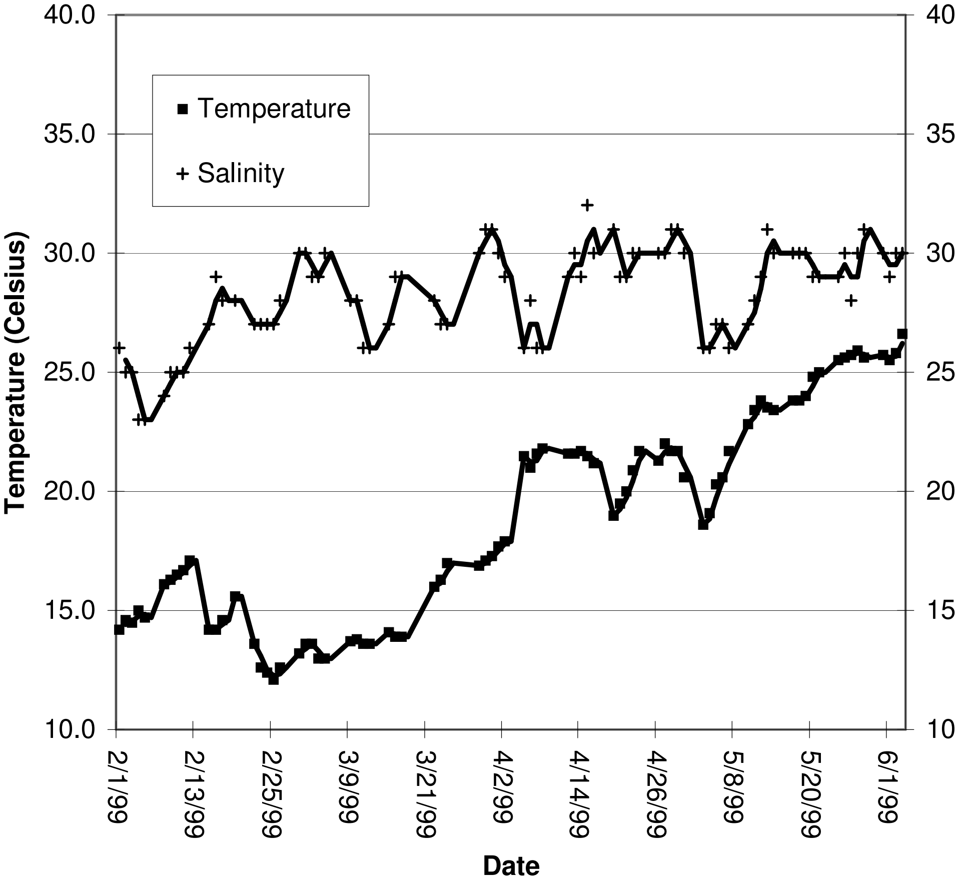

Figure 2.2 – Water temperature and salinity of the Skidaway River for the duration of the diet experiment, February 1 – June 3, 1999. Individual tanks received water from the same source at the same flow rate. Data were typically collected five days a week and a moving average trendline was applied to the graph to better illustrate the data.

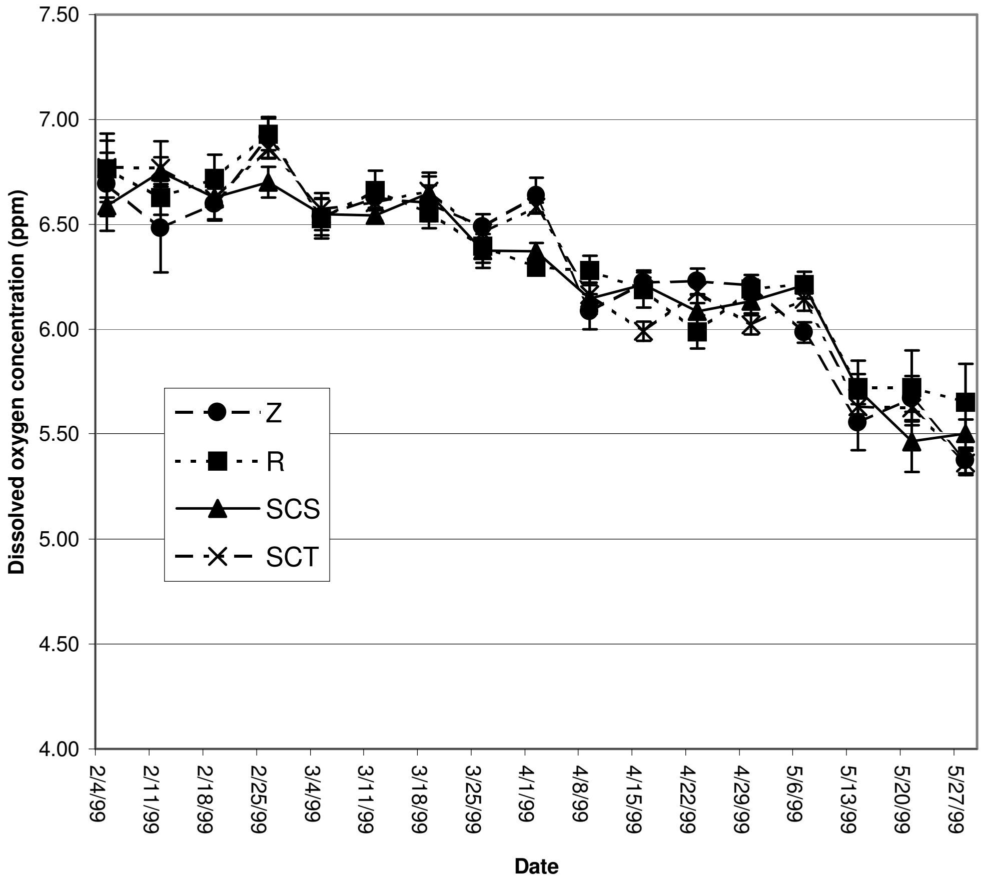

Figure 2.3 – Mean dissolved oxygen concentration (± SE) of each treatment for the duration of the diet experiment, February 1 – June 3, 1999. Measurements were taken at least weekly and values of each replicate tank were averaged for each treatment.

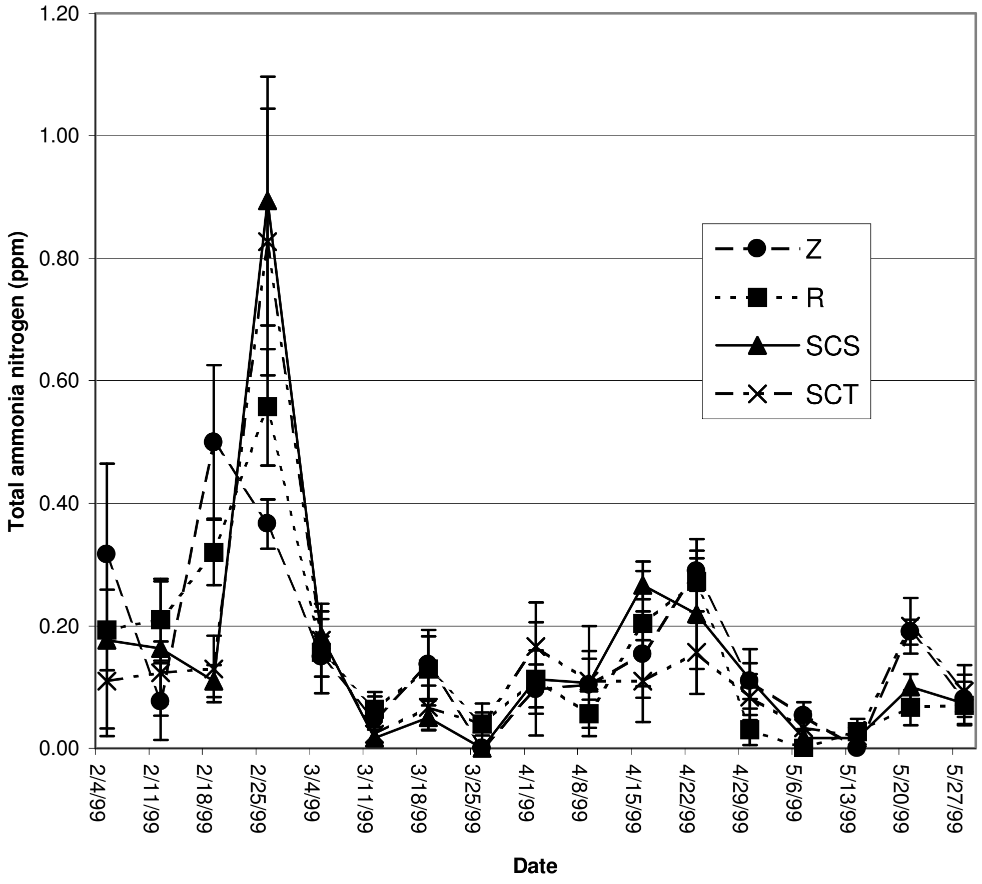

Figure 2.4 – Mean ammonia (TAN) concentration (± SE) of each treatment for the duration of the diet experiment, February 1 – June 3, 1999. Measurements were taken at least weekly and values of each replicate tank were averaged for each treatment.

Feeding Ration Experiment

In the feeding ration experiment (Fig. 2.5), significant differences ($p = 0.0028$) in weight of fish were found after 6 weeks. By week 10, Duncan's test ($\propto = 0.05$) showed that fish fed daily rations of 7.5% (8.22 ± 0.49 g) and 5.0% (8.01 ± 0.48 g) were not significantly different in weight. Both of these treatments produced larger fish than the 2.5% (6.69 ± 0.43 g) daily ration treatment.

Growth and feed conversion data are presented in Table 2.3. Weight gain, RGR, SGR, and DWG were all highest for fish fed a 7.5% daily ration. Feed conversion ratio was lowest for fish fed a 2.5% daily ration.

Mortality for each treatment is as follows, 18.2% ± 1.9% for the 2.5% feeding ration, 10.2% ± 2.8% for the 5.0% feeding ration, and 12.4% ± 1.6% for the 7.5% feeding ration. There were no significant differences ($p = 0.1710$) in mortality among treatments.

Water quality data for this experiment are presented in Figures 2.6 – 2.8. Temperature ranged from 17.4º C – 27.9º C, salinity ranged from 26 – 36 ppt, dissolved oxygen ranged from 5.00 – 6.10 ppm and TAN ranged from 0.00 – 0.62 ppm throughout the experiment. No graph was generated for pH because the value was virtually constant ($\approx 7.5 - 7.8$) throughout the experiment.

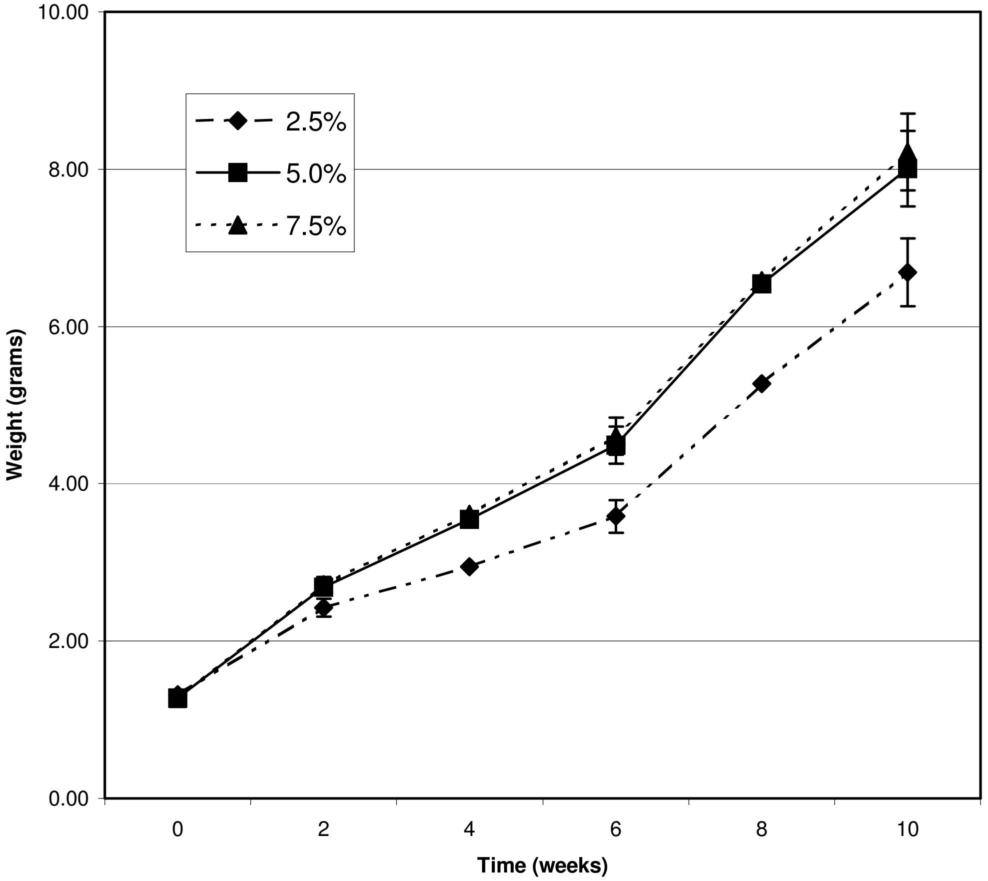

Figure 2.5 – Mean weight (± SE) versus time for juvenile black sea bass fed 2.5%, 5.0% and 7.5% daily rations (dry weight of food / wet weight of fish) of Zeigler Salmon Starter feed. At weeks 4 and 8, weights of individual fish were not obtained, as was done at all other intervals. Therefore at weeks 4 and 8, computation of standard error was not possible and no error bars are given for these time intervals. At week 2, standard errors were very small and therefore error bars are difficult to distinguish in the graph.

Table 2.3 – Initial and final weights, weight gain, relative growth rate (RGR), specific growth rate (SGR), daily weight gain (DWG), feed conversion ratio (FCR), and adjusted feed conversion ratio (FCR_{adj}) for juvenile black sea bass fed daily rations of 2.5%, 5.0% and 7.5%.

	Variable							
Daily feeding ration	Initial weight (g)	Final weight (g)	Weight gain (g)	RGR (%)	SGR (%/day)	DWG (g/day)	FCR	FCR_{adj}
2.5%	1.32 ± 0.04	6.69 ± 0.43	5.37	406.8	2.32	0.077	0.95	0.90
5.0%	1.28 ± 0.03	8.01 ± 0.48	6.73	525.8	2.62	0.096	1.77	1.74
7.5%	1.27 ± 0.04	8.22 ± 0.49	6.95	547.2	2.67	0.099	2.71	2.64

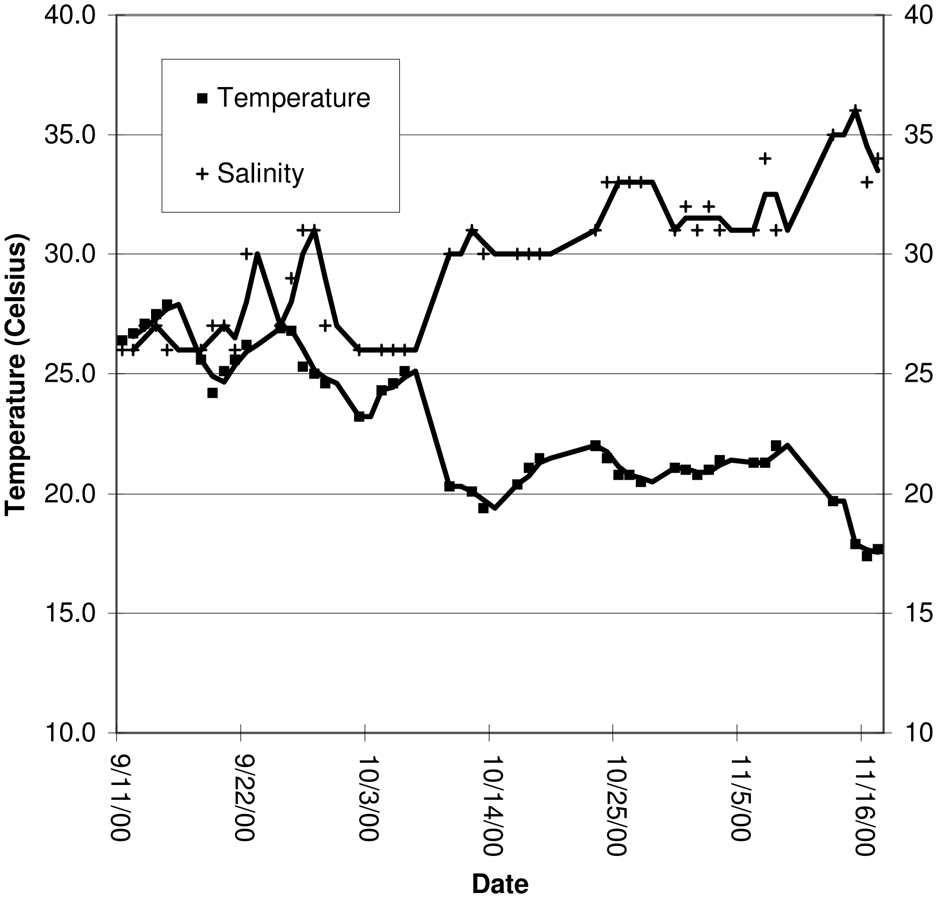

Figure 2.6 – Water temperature and salinity of the Skidaway River for the duration of the feeding ration experiment, September 8 – November 17, 2000. Individual tanks received water from the same source at the same flow rate. Data were typically collected five days a week and a moving average trendline was applied to the graph to better illustrate the data.

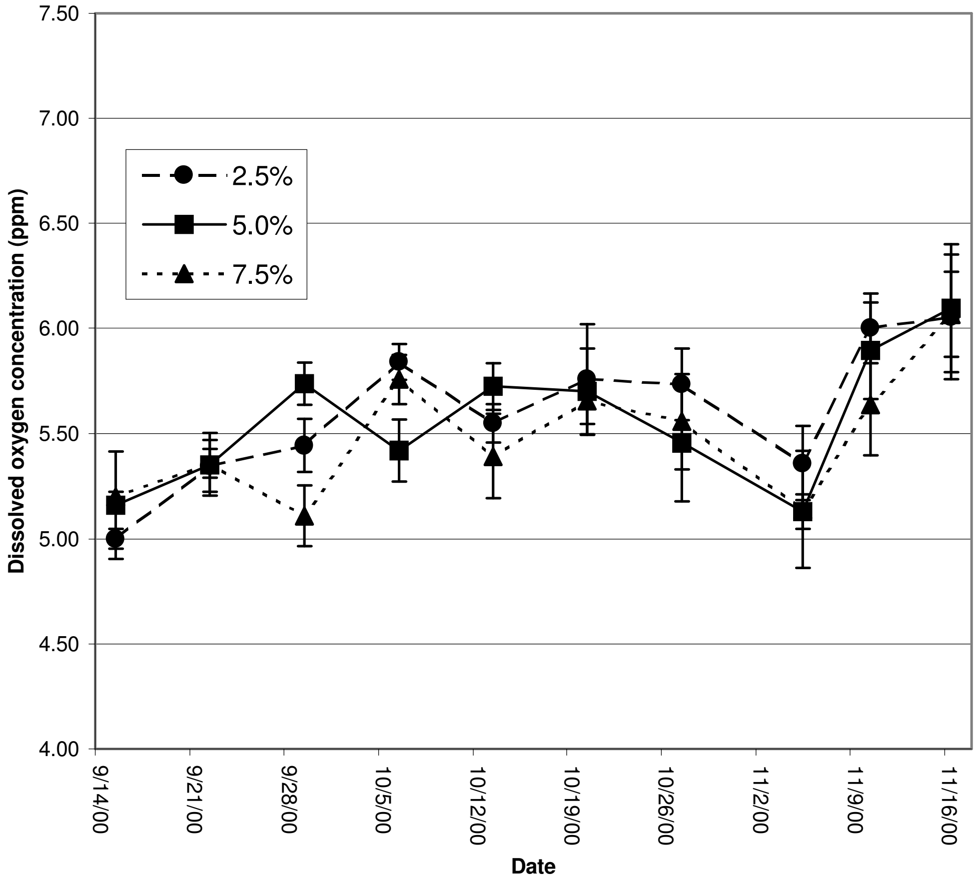

Figure 2.7 – Mean dissolved oxygen concentration (± SE) of each treatment for the duration of the feeding ration experiment, September 8 – November 17, 2000. Measurements were taken at least weekly and values of each replicate tank were averaged for each treatment.

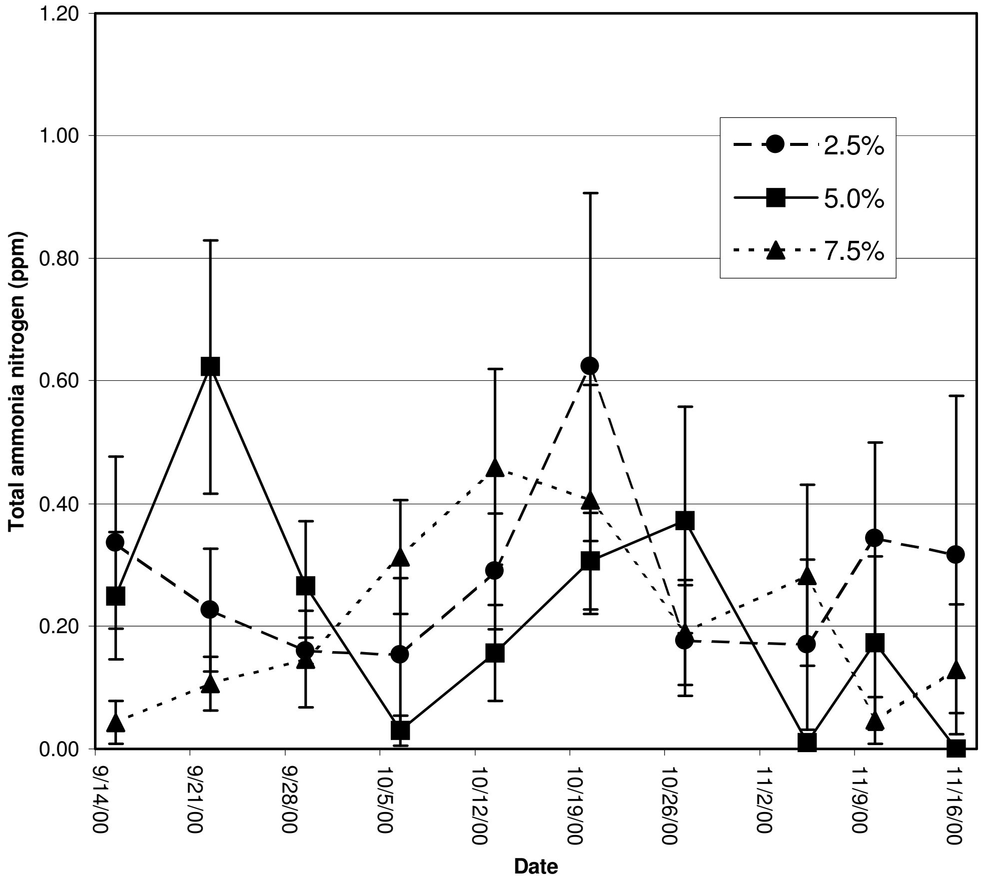

Figure 2.8 – Mean ammonia (TAN) concentration (± SE) of each treatment for the duration of the feeding ration experiment, September 8 – November 17, 2000. Measurements were taken at least weekly and values of each replicate tank were averaged for each treatment.

Discussion

Diet Experiment

The diet experiment shows that juvenile black sea bass of the size used in this experiment grow best on a high protein (≈ 50%) commercial feed, with diet Z producing larger fish than diets R, SCS, and SCT. This result supports similar diet studies with juvenile and adult black sea bass. Berlinsky et al. (2000) recorded the best growth rates of juvenile black sea bass (15-30 mm, TL) with diets containing 55% protein (Biokyowa B-Series Fry and Corey Hi-Pro Fry).

Walker and Moroney (2000) found that adult black sea bass fed a salmon feed (45% protein, 26% lipid) grew significantly larger than fish fed a trout feed (38% protein, 8% lipid) with lower protein and lipid composition than the salmon feed. Similarly, Berlinsky et al. (2000) found that a 52% protein, 18% lipid feed (Corey Clear Choice) produced significantly larger fish than a 38% protein, 12% lipid feed (Zeigler Trout Grower) for sub-adult black sea bass. Copeland et al. (2001) also reported higher growth rates in sub-adult black sea bass with commercial feeds containing 50 – 56% protein when compared to commercial feeds containing 41 – 45% protein.

Investigations of protein requirements in commercial feeds for other marine fishes have yielded similar results. European sea bass *Dicentrarchus labrax* (Perez et al. 1997; Peres and Oliva-Teles 1999), striped bass *Morone saxatilis* (Millikin 1982), and estuary grouper *Epinephelus salmoides* (Teng et al. 1978) have been shown to grow optimally with diets containing at least 50% protein. Furthermore, Thoman et al. (1999) report a minimum protein level of 44% for red drum *Sciaenops ocellatus*.

Both the Zeigler and Rangen feeds contain higher protein and lipid levels than either of the Silver Cup feeds, so the higher growth rates achieved by diets Z and R were not surprising. Interestingly, the Zeigler feed produced larger fish than the Rangen feed even though they have approximately the same nutritional composition of protein and lipid (Table 2.1). Berlinsky et al. (2000) indicate that high protein content is more beneficial than high lipid content in a commercial feed for black sea bass. This is expected as proteins provide the essential amino acids necessary for growth. Additionally, the storage of lipid in fish translates to lower ultimate weight gain than the storage of proteins. According to Jobling (1994), 1 gram of protein can bind as much as 3 to 4 grams of water, thus further increasing weight gain with consumption of protein. Therefore, replacing excessive lipid in an artificial black sea bass diet with more protein should yield better growth rates than the salmon feeds used in these experiments. Ellis et al. (1996) report a FCR of 0.94 for juvenile Nassau grouper (*Epinephelus striatus*) fed a commercial diet with 55.6% protein and 8% lipid levels. This is much better than the FCR values recorded in the present experiment. A feed with this nutritional composition will presumably produce better growth rate and FCR in black sea bass than the feeds tested in the present experiment. This feed will cost more than the feeds tested here, because of the increased protein, the most expensive ingredient in fish feed. Ellis et al. (1996) also report that excessive dietary lipid can actually suppress appetite and reduce feed intake and growth. Similarly, Johnson et al. (2002) found that growth rates of juvenile Nassau grouper were negatively correlated to dietary lipid levels of 6%, 9%, 12% and 15%.

The carbohydrate composition of diet Z (15%) is higher than that of diet R (8%), which could account for the difference in growth. Carbohydrate can have a sparing effect on protein and a level of 15-18% carbohydrate has been recommended for juvenile marine fishes of the Percoidea super-family (New 1986), in which *C. striata* is included. Higher carbohydrate levels, however, can have a negative effect on both growth and lipid deposition in the carcass. An ideal commercial feed for black sea bass should therefore have a composition of high protein (> 50%), low lipid (5 – 10%) and moderate carbohydrate (approximately 15%).

In this experiment, the ingredients are slightly different in the Zeigler and Rangen diets, although the protein and lipid compositions are the same. Just as important as the percentage composition of protein and lipid are the sources of those constituents. The Zeigler feed may have contained more fish protein than the Rangen diet. Although both diets contain 50% protein, some of that protein is of animal origin and the rest is of plant origin. Protein of animal origin, particularly fish meal, is more readily assimilated by fish and therefore yields better growth rates than protein of plant origin (Jobling 1994).

There are many high protein, high lipid diets available for salmonid fishes, but few commercial diets exist for strictly marine fishes. Zeigler has recently introduced a flounder diet (55% protein and 12% lipid) that will most likely be a good choice for black sea bass. Many feed manufacturers have indicated that they will customize a feed formula for a fish farmer, provided that the buyer purchase a minimum predetermined amount, usually a ton or more of feed. This amount is unfortunately excessive for many of the small-scale aquaculturists, who may be in the experimental stage of black sea bass aquaculture. As the aquaculture industry continues to grow and new species are cultured here in the United States, the availability and quality of feeds for marine fish will certainly increase.

Mr. Gary Kinard, one of the active black sea bass fish farmers operating in nearby McIntosh County, has been utilizing various trout feeds (40-45% protein, 7-10% lipid, personal communication). The results of this experiment suggest that the growth rate of his black sea bass can be increased by as much as threefold by changing from a trout feed to a salmon feed. This is based on the comparison of growth rates of fish fed Zeigler Salmon Starter and Nelson's Silver Cup Trout Crumbles.

Fish mortality for this experiment was high because of the small stocking size (0.82 ± 0.02 g) of the fish. Therefore, aquaculturists should purchase larger black sea bass fingerlings, at a size of approximately 3-5 grams. At a mean size of 0.8-g, cannibalism is high and not all fish seem to be properly weaned to pelletized or crumbled feed. Cannibalism is a problem that has been previously observed among black sea bass and other serranids. Roberts et al. (1976) report high incidence of cannibalism in 24 DPH (days post hatch) black sea bass larvae. Johnson et al. (2002) list reports of cannibalism in estuary grouper (*Epinephelus salmoides*), malabar grouper (*E. malabaricus*), and greasy grouper (*E. tauvina*). They also cite cannibalism as the primary cause of mortality in juvenile Nassau grouper (*E. striatus*) of their experiments. In their review of cannibalism in larviculture, Hecht and Pienaar (1993) explain that smaller fry can become stressed by the presence of larger cannibalistic conspecifics. This can lead to reduced growth rates and susceptibility to disease. In our experiment, many fish that were stocked at sizes smaller than 0.5 g never seemed to accept an artificial feed, and they appeared emaciated for weeks after stocking. Eventually these fish either succumbed to infection of some kind or were cannibalized. Larger juvenile black sea bass (approximately 9 grams) have been used for subsequent experiments investigating optimal temperature and salinity for growth of black sea bass (Chapter 3). These fish exhibited much greater hardiness, survival, appetite, and growth rates.

Near the end of the experiment (week 14), three tanks of fish were killed because of equipment failure. This loss obviously contributed to the high mortality and is responsible for the large standard error values associated with the reported mortality statistics. Notwithstanding this loss, mortality was high in this experiment. At week 10, before the aforementioned fish kill, mortality was 28.7% ± 7.6% for diet Z, 18.0% ± 3.8% for diet R, 33.3% ± 16.9% for diet SCS, and 36.0% ± 8.4% for diet SCT.

Growth rates increased substantially after week 10 (Figure 2.1). After this time, the temperature of the Skidaway River, the water source for the flow-through tanks used in this experiment, began to increase. The experiment commenced on February 1, 1999 when river temperature was 14° C (Figure 2.2). At week 10, on April 7, river temperature was 22° C. By the end of the experiment, June 3, 1999, river temperature had reached 27° C. The effect of temperature on growth of juvenile black sea bass has been investigated in a separate experiment (Chapter 3). This experiment found significant differences in growth of fish at 15° C, 20° C and 25° C, with negative growth observed in the 15° C treatment.

Feeding Ration Experiment

The feeding ration experiment shows that a 5.0% daily ration produces the same size fish as a 7.5% daily ration, and both produced larger fish than a 2.5% daily ration.

Considering cost of feed and FCR, a daily feeding ration of 5.0% would be preferable to a 7.5% ration. This agrees with Kim's (1987) theoretical calculation of an optimal daily feeding ration of 4.76%, based on satiation feeding.

These results are critical for the aquaculture industry as it struggles to find the economic equilibrium of intensive growth and operating cost. One could feed fish a 5.0% ration to increase growth, but this also doubles feed cost of a 2.5% ration. Since fish fed a 5.0% ration were not twice as large as the fish fed a 2.5% ration in the course of this experiment, one might not want to incur the extra feed cost for such an increase in growth. Other operational costs, such as electricity and labor, might justify the increased growth rates with a higher feeding ration. In this case, a 5.0% ration might be preferable to a 2.5% ration. The cost of feed in an aquacultural facility is usually the highest expenditure of the business, often comprising 50% of the total operational cost of the facility (Avault 1996). Therefore, great care must be taken to ensure that the farmer achieves optimal growth for the amount of feed expended. On a large, industrial scale the difference between a 2.5% and a 5.0% feeding ration could equate to thousands of dollars. The farmer must be certain that this increase in growth rate is worth the expenditure.

Feeding ration is also a function of the type of diet (feed) given to the fish. The optimal feeding ration determined in this experiment would presumably hold true for a diet of similar nutritional composition. If one were using a cheaper diet of lower lipid and protein composition, one could reasonably assume that a higher ration of feed will be required to maximize growth. Optimal feeding ration is also a function of environmental conditions. Boehlert and Yoklavich (1983) showed that lower temperature yields lower optimal feeding ration for juvenile black rockfish (*Sebastes melanops*). To fully exploit the concept of "optimal feeding ration", more work needs to be done to explore the cumulative effects of environmental conditions on feeding ration and ultimately develop a dynamic feeding model for black sea bass.

Fish mortality was lower for the feeding ration experiment (14%) than for the diet experiment (69%) presumably because larger fish (1.3 g vs. 0.8 g) were stocked at the beginning of the experiment. Fish of this size are apparently less susceptible to cannibalism, are properly weaned to an artificial diet, and are better able to fight infectious diseases. In the feeding ration experiment, very few fish exhibited the emaciated condition that was observed in the diet experiment. As mentioned previously, fish farmers should purchase fingerlings of a slightly larger size, approximately 3-5 grams, to achieve optimal survival and grow-out potential.

During both experiments, water quality was never problematic (Figures 2.2 – 2.4, 2.6 – 2.8). Ammonia levels were recorded occasionally at levels above 1 ppm in both experiments, but these levels never seemed to affect the fish. Temperature and salinity levels were typical for Georgia estuaries at the time of year these experiments were conducted. In subsequent experiments with juvenile black sea bass (Chapter 3), ammonia levels as high as 3.5 ppm have been recorded, again without noticeable deleterious effects to the fish. Additionally, Hoff (1970) noted that the fish in his experiments experienced dissolved oxygen levels as low as 3 ppm for periods of 1 – 3 days "with no apparent effect" on the fish. These observations support the claim that black sea bass is a suitable species for intensive aquaculture.

Black sea bass have exhibited hardiness, excellent market potential, fast growth and tolerance of inferior water quality (Hoff 1970; Roberts et al. 1976; Harpster et al. 1977; Tucker 1984; Kim 1987; Costa and Provenzano 1993; Berlinsky et al. 2000; Kupper et al. 2000; Walker and Moroney 2000). Black sea bass also quickly adapt to a tank environment and when properly weaned will readily accept a pelletized food (Hoff 1970; Kim 1987; Berlinsky et al. 2000). With this high resilience, adaptability, and desirable market value, this fish offers great potential for mariculture.

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CHAPTER 3

EFFECTS OF TEMPERATURE AND SALINITY ON GROWTH OF JUVENILE BLACK SEA BASS *CENTROPRISTIS STRIATA* WITH IMPLICATIONS FOR AQUACULTURE[2]

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Abstract

Black sea bass, *Centropristis striata* L., has recently gained popularity in the live seafood markets of the northeast United States. Currently, a few small-scale operations have made rudimentary attempts to culture this species commercially. Fish farmers cite the lack of data pertaining to the grow-out of black sea bass as the primary reason for their hesitant attitude toward culturing this species. Among the needed information for grow-out of black sea bass is the optimal temperature and salinity for growth. In this study, optimal temperature and salinity were determined experimentally for growth of juvenile black sea bass (approximately 9.2 grams). The temperature experiment compared growth at temperatures of 15° C, 20° C, 25° C and 30° C and the salinity experiment compared growth at 10 ppt, 20 ppt, and 30 ppt salinities. Both of these experiments were performed in closed aquaria. By the end of the temperature experiment (6 weeks), Analysis of Variance (ANOVA) found significant differences ($p < 0.0001$) in weight among treatments. Tukey's studentized range test ($\propto = 0.05$) showed that fish grown at 25° C were significantly larger than those grown at 20° C and 30° C. All of these temperatures produced significantly larger fish than those grown at 15° C. By the end of the salinity experiment (12 weeks), ANOVA found significant differences ($p < 0.0001$) in weight among treatments. Tukey's studentized range test ($\propto = 0.05$) showed that fish grown in salinities of 20 ppt and 30 ppt did not produce significantly different weights in fish. Both of these salinities produced significantly larger fish than a salinity of 10 ppt. The results of these experiments will aid fish farmers in balancing optimal growth with operating costs of the aquacultural facility.

Keywords: Centropristis striata, aquaculture, temperature, salinity, growth

Introduction

Black sea bass, *Centropristis striata* L., is a commonly sought fish throughout its range from Florida to Massachusetts (Musick and Mercer 1977). Recreational anglers harvest more black sea bass annually than commercial anglers. For the period of 1979-1990, recreational fishing accounted for 43% (by weight) of landings, while commercial landings accounted for 33% and headboat landings accounted for 24% of the total harvest of black sea bass (Vaughan et al. 1995).

A developing market for sushi grade black sea bass is offering desirable prices for live fish. Many fishers have chosen to seek the higher prices offered by the live market than by the traditional fish markets along the coast of Georgia. Currently in Georgia, fishers are offered approximately $5.50 per kilogram for whole fish (Charlie Phillips, personal communication). Live black sea bass, however, can be transported to markets in the northeast United States and sold for $11.00-$17.60 per kilogram (Gary Kinard, personal communication). Berlinsky et al. (2000) report that the demand for black sea bass in the northeast United States usually exceeds supply, thereby making this area a lucrative market for live fish.

Vaughan et al. (1995) report that catch per unit effort (CPUE) for black sea bass has declined in the South Atlantic Bight. New size regulations make the practice of trapping black sea bass more difficult as fishers find it harder to catch great numbers of minimum-legal-sized fish. This research strives to satisfy demand and support an emerging aquacultural industry, while protecting wild stocks of black sea bass. These experiments are part of an ongoing effort to develop economically viable methods for mariculture of black sea bass, using hatchery-reared fingerlings instead of wild-trapped adults and sub-adults.

Despite a sizable volume of literature pertaining to black sea bass aquaculture, commercial culture of this species has yet to fully develop. Previous research on black sea bass mariculture includes studies of brood stock management and spawning techniques (Hoff 1970; Roberts et al. 1976; Tucker 1984; Chappell et al. 2001; Stuart et al. 2001), larval development and culture (Hoff 1970; Roberts et al. 1976; Tucker 1984; Berlinsky et al. 2000), grow-out of juveniles, (Harpster et al. 1977; Kim 1987; Berlinsky et al. 2000; Atwood et al. 2001), and grow-out of sub-adults and adults (Kim 1987; Costa and Provenzano 1993; Berlinsky et al. 2000; Kupper et al. 2000; Walker and Moroney 2000; Copeland et al. 2001). Previous research involving the effects of salinity and temperature during grow-out of juvenile black sea bass is of particular interest in this study. Berlinsky et al. (2000) investigated the effect of salinity on growth of juvenile black sea bass as well as the effect of temperature on growth of larvae. Atwood et al. (2001) investigated minimum and maximum tolerances of salinity and temperature for juvenile black sea bass.

This work was intended to build upon the studies of grow-out of juvenile black sea bass by experimentally determining the optimal temperature and salinity for this species. This research should serve as a starting point upon which to build an understanding of the salinity and temperature requirements throughout the life stages of this fish. Understanding how other environmental and physiological factors affect these important growth parameters will be important.

Methods

Brood stock were captured by pot traps off the coast of Virginia and later subjected to hormone induced spawning to produce the fingerlings used in the temperature and salinity experiments. These fingerlings (mean weight = 1.29, SE = ± 0.02 g) were purchased in September 2000 from Southland Fisheries Corporation in Edisto, SC. Fish were kept for three months in 600-L fiberglass holding tanks with a flow-through circulation of estuarine water from the Skidaway River and supplemental aeration was provided by airstones. During the holding period, fish were fed Zeigler Salmon Starter (size #2 and #3 crumble) daily to apparent satiation.

Temperature experiment

Fish (9.0 ± 0.16 g) were raised for 42 days in 76-L closed glass aquaria in four climate-controlled rooms. Temperatures in each room (and thus water temperatures) were maintained at 15° C, 20° C, 25° C and 30° C, respectively. Fish were kept under constant light (30 lx) and provided with supplemental aeration by airstones. Tanks were equipped with an 'undergravel' type of biofiltration system. Prior to the experiment, conditioning of the biofilter of each aquarium was performed according to Moe (1992). Fish were fed a floating pellet (Rangen XTR 450, 2.4 mm pellet, 45% protein, 16% lipid) at a 3.0% daily ration (grams dry weight of food/grams wet weight of fish). Twelve tanks were stocked with 30 fish per tank. A random number table was used to assign treatments (n=4) and replicates (n=3) to each batch of 30 fish immediately prior to stocking. All fish from each tank were weighed and tanks were cleaned biweekly. Fish were individually weighed in a tared, 1-L beaker of seawater on an Acculab V-1200 balance. Fish were not anesthetized during weighing. After each weighing, new rations were calculated and adjusted for each tank. Treatment means (weight) were analyzed by Analysis of Variance (ANOVA) and Tukey's studentized range test with The SAS System for Windows v6.12 software. Mortality data (expressed in decimal form) were arcsine transformed for each replicate. The transformed data were then analyzed by ANOVA using Microsoft Excel 2000 software. Relative growth rate (RGR), specific growth rate (SGR), and daily weight gain (DWG), were calculated using Formulas 3.1-3.3, respectively.

(3.1) RGR = 100 X (final weight – initial weight)/(initial weight)

(3.2) SGR = 100 X [ln (final weight) – ln (initial weight)]/(time in days)

(3.3) DWG = (final weight – initial weight)/(time in days)

Feed conversion ratio was calculated using Formula 3.4. To account for weight loss attributed to mortality, an adjusted FCR was calculated using Formula 3.5.

(3.4) FCR = (weight of feed consumed)/(final total biomass – initial total biomass)

(3.5) FCR_{adj} = (weight of feed consumed)/[(final total biomass) – (number of fish remaining at the end of the experiment) X (initial mean weight)].

Estuarine water withdrawn from the Skidaway River was sterilized with 12.5% sodium hypochlorite and any excess chlorine was neutralized with sodium thiosulfate the following day. Water sterilized in this manner was used for initial filling of aquaria and subsequent water exchanges (approximately 80%) at least twice a week for all tanks. Salinities ranged from 27-34 ppt throughout the experiment and varied according to ambient salinity in the Skidaway River at the time of the water withdrawal. All tanks were given water of equal salinity during water changes and salinity never changed by more than 3 ppt after any single water change. Water temperature, salinity, dissolved oxygen, ammonia concentration, and pH were measured at least weekly. Dissolved oxygen was measured with an Orion (model 830) dissolved oxygen meter and pH was measured with an Oakton (model pH Testr2) pH meter. Salinity and temperature were measured with a refractometer and alcohol thermometer (respectively). Total ammonia nitrogen (TAN) was measured with a LaMotte colorimeter (model SMART), which uses an adaptation of the Nesslerization method.

Salinity experiment

Fish (9.4 ± 0.30 g) were raised for 81 days in 114-L closed glass aquaria, each assigned a salinity of 10 ppt, 20 ppt, or 30 ppt. Tanks were kept in a climate-controlled room and temperature was maintained at approximately 22° C throughout the experiment. Fish were kept under constant light (30 lx) and provided with supplemental aeration by airstones. Tanks were equipped with an "undergravel" type of biofiltration system. Prior to the experiment, conditioning of the biofilter of each aquarium was performed according to Moe (1992). Nine tanks were stocked with 30 fish per tank. A random number table was used to assign treatments (n=3) and replicates (n=3) to each batch of 30 fish prior to stocking.

Each batch of fish was incrementally acclimated to their assigned salinity over the course of four days prior to the start of the experiment. Ambient salinity of the flow-through system in which the fish were held prior to stocking was 32 ppt at the time of stocking. Salinities of three reservoirs (10 ppt, 20 ppt and 30 ppt) were adjusted by diluting estuarine water from the Skidaway River. The diluted water was then sterilized as in the temperature experiment. This sterilized water was used for initial filling of aquaria and subsequent water exchanges (approximately 80%) at least twice a week for all tanks. Salinity of the Skidaway River never dropped below 30 ppt during the course of the experiment, so the addition of artificial salt was never necessary. Fish were fed a floating pellet, (Rangen XTR 450, 2.4 mm pellet, 45% protein, 16% lipid) at a 3.0% daily ration. Fish were weighed and tanks were cleaned in the same manner as the temperature experiment. Treatment means (weight), mortality data and feeding ration were calculated and analyzed as in the temperature experiment. Growth rates (RGR, SGR, and DWG) and feed conversion ratio (FCR and FCR_{adj}) were calculated as in the temperature experiment.

Water temperature, salinity, dissolved oxygen, ammonia concentration, and pH were measured at least weekly in the same manner as the temperature experiment.

Results

Temperature

In the temperature experiment (Fig. 3.1), significant differences ($p < 0.0001$) in weight of fish were found after 2 weeks, with temperatures of 20º C, 25º C and 30º C producing larger fish than 15º C. At weeks 4 and 6, Tukey's studentized range test ($\propto = 0.05$) showed that fish grown at 25º C were larger than those grown at 20º C and 30º C. All of these temperatures produced significantly larger fish than those grown at 15º C. Fish grown at 20º C and 30º C were not significantly different in size.

Weight gain, RGR, SGR, and DWG were highest in the 25º C treatment (Table 3.1). Similarly, FCR was lowest in the 25º C treatment.

Mortality for each treatment after six weeks was 12.2% ± 2.9% at 15º C, 13.3% ± 1.9% at 20º C, 12.2% ± 1.1% at 25º C, and 32.2% ± 3.3% at 30º C. Significant differences ($p = 0.0020$) in mortality were found among treatments. Tukey's studentized range test ($\propto = 0.05$) showed that mortality was significantly higher in the 30º C treatment when compared to all other treatments. No significant differences in mortality were found among the 15º C, 20º C, and 25º C treatments.

Water quality data for this experiment are presented in Figures 3.2 and 3.3. Dissolved oxygen ranged from 4.23 – 6.70 ppm and varied by treatment due to the effect of temperature on oxygen saturation. TAN ranged from 0.00 – 1.17 ppm throughout the experiment. No graph was generated for pH or salinity because the values were virtually constant (pH ≈ 7.2 – 8.0, salinity ≈ 27 – 34 ppt) throughout the experiment. Similarly, temperature was not graphed because the temperature in each climate-controlled room was constantly maintained throughout the experiment.

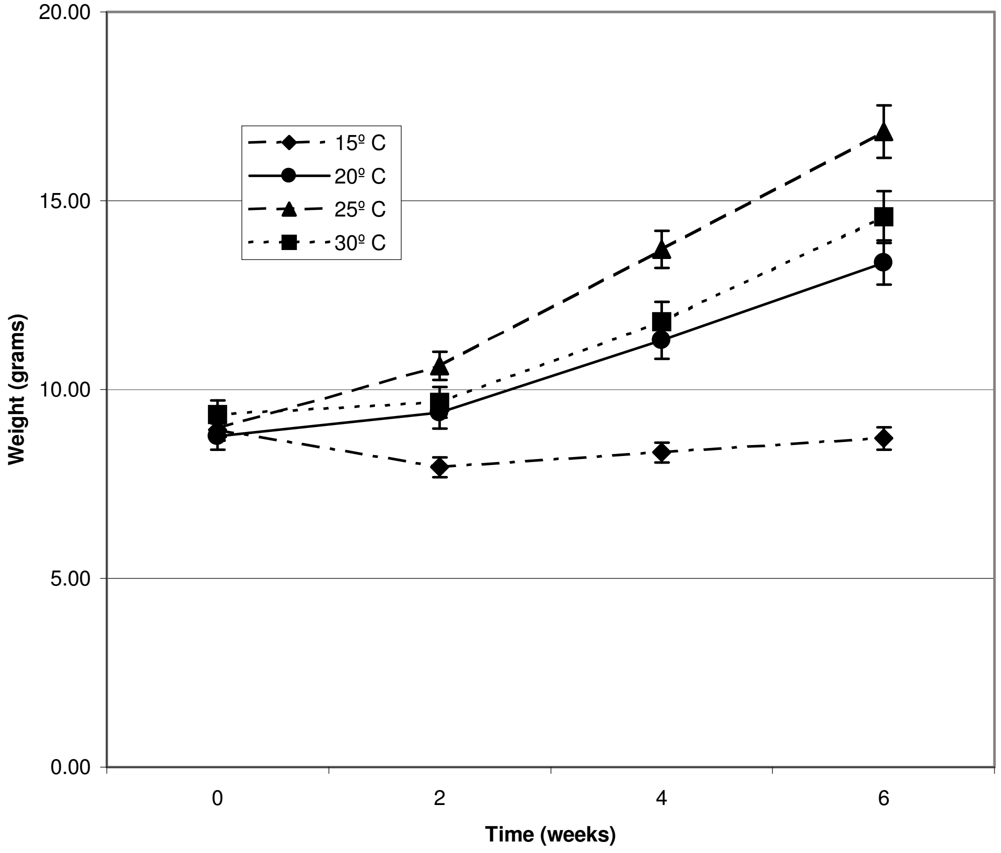

Figure 3.1 – Mean weight (± SE) versus time for juvenile black sea bass grown in closed tanks at temperatures of 15º C, 20º C, 25º C and 30º C.

Table 3.1 – Initial and final weights, weight gain, relative growth rate (RGR), specific growth rate (SGR), daily weight gain (DWG), feed conversion ratio (FCR), and adjusted feed conversion ratio (FCR_{adj}) for juvenile black sea bass grown in closed tanks at temperatures of 15º C, 20º C, 25º C and 30º C.

	Variable							
Temperature	Initial weight (g)	Final weight (g)	Weight gain (g)	RGR (%)	SGR (%/day)	DWG (g/day)	FCR	FCR_{adj}
15º C*	8.94 ± 0.28	8.71 ± 0.30	– 0.23	– 2.6	– 0.06	– 0.005	– 5.62	– 36.09
20º C	8.76 ± 0.34	13.36 ± 0.59	4.60	52.5	1.00	0.110	2.86	2.02
25º C	8.96 ± 0.29	16.83 ± 0.70	7.87	87.8	1.50	0.187	1.57	1.32
30º C	9.34 ± 0.38	14.57 ± 0.68	5.23	56.0	1.06	0.125	14.84	2.23

* Fish in the 15º C treatment lost weight over the course of the 6-week experiment, producing the negative FCR and growth rates.

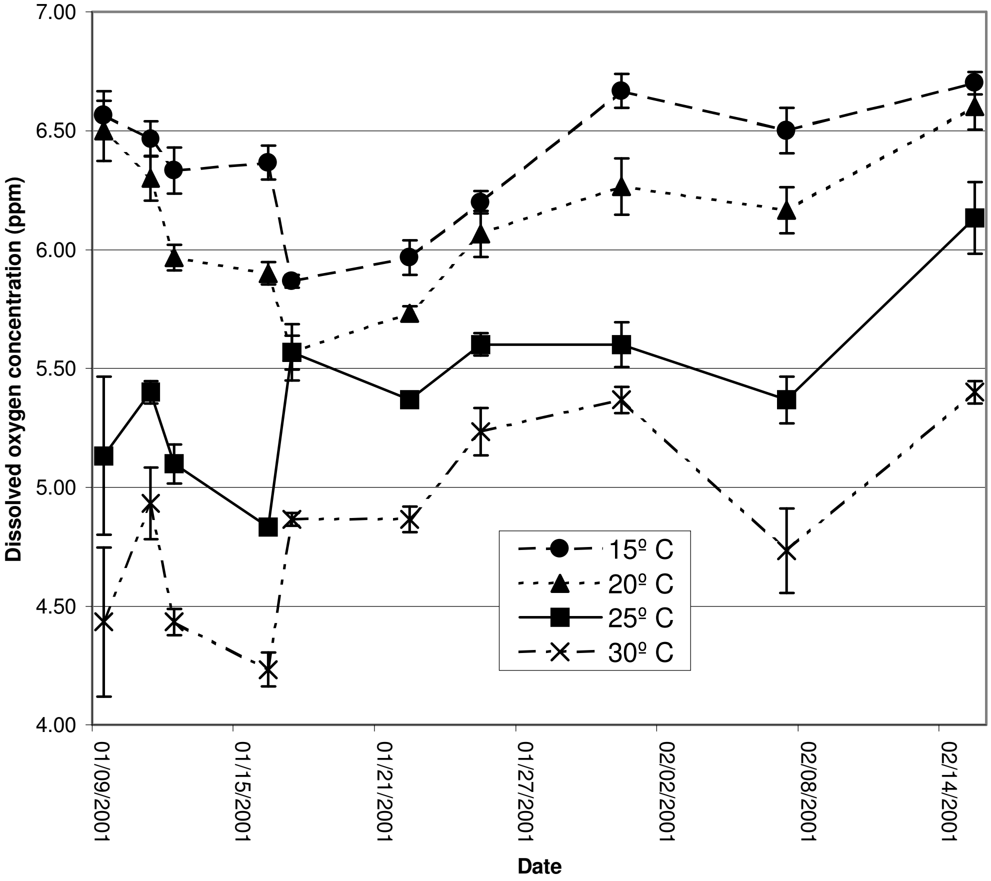

Figure 3.2 – Mean dissolved oxygen concentration (± SE) of each treatment for the duration of the temperature experiment, January 5 – February 16, 2001. Measurements were taken at least weekly and values of each replicate tank were averaged for each treatment.

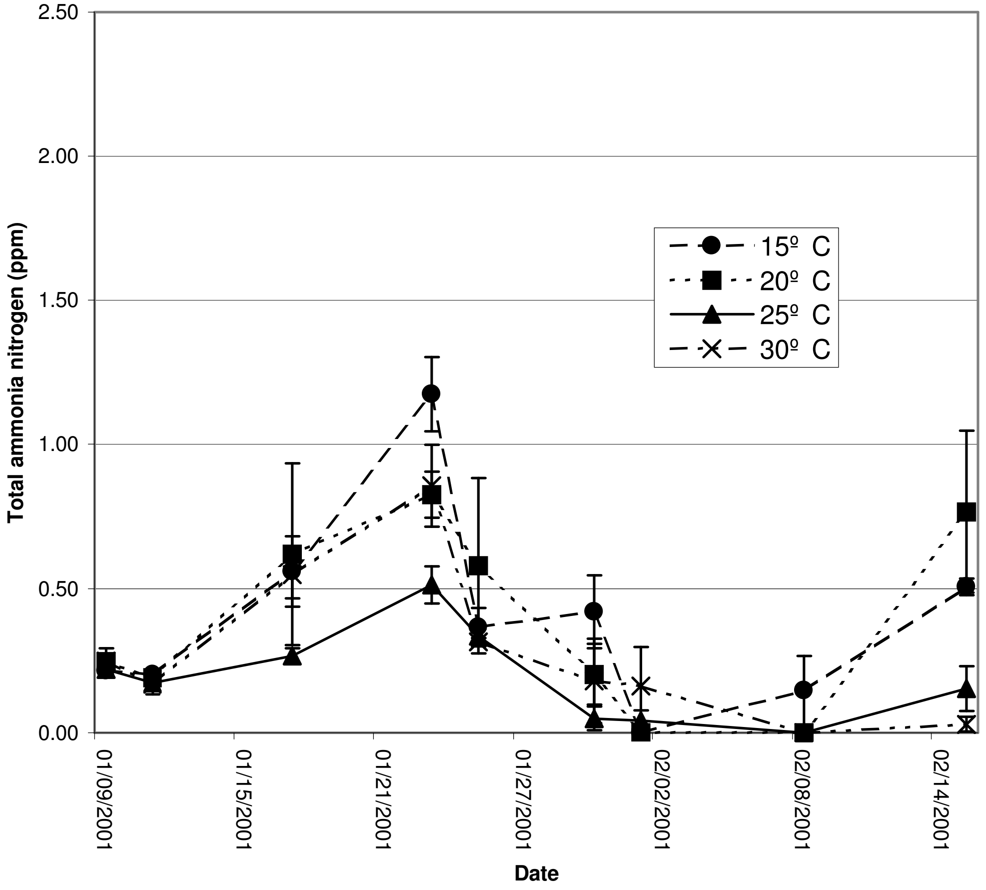

Figure 3.3 – Mean ammonia (TAN) concentration (± SE) of each treatment for the duration of the temperature experiment, January 5 – February 16, 2001. Measurements were taken at least weekly and values of each replicate tank were averaged for each treatment.

Salinity

In the salinity experiment (Fig. 3.4), significant differences ($p < 0.0001$) in weight of fish were found after 4 weeks, with salinities of 20 ppt and 30 ppt producing larger fish than 10 ppt. From week 4 until the end of the experiment (week 12), Tukey's studentized range test ($\propto = 0.05$) showed that fish grown in salinities of 20 ppt and 30 ppt produced equivalent weights in fish. Both of these salinities produced significantly larger fish than a salinity of 10 ppt.

Growth and feed conversion data are presented in Table 3.2. Weight gain, RGR, SGR, and DWG were highest in the 20 ppt treatment. FCR was lowest in the 30 ppt treatment, but FCR_{adj} was lowest in the 20 ppt treatment.

Mortality for each treatment after 81 days was 41.1% ± 2.9% at 10 ppt, 21.1% ± 8.0% at 20 ppt, and 14.4% ± 1.1% at 30 ppt. Significant differences ($p = 0.0209$) in mortality were found among treatments. Tukey's studentized range test ($\propto = 0.05$) showed that mortality was significantly higher for fish grown at 10 ppt when compared to 30 ppt. Mortality in the 20 ppt treatment was not significantly different than the other treatments.

Water quality data for this experiment are presented in Figures 3.5 and 3.6. Dissolved oxygen ranged from 5.20 – 6.43 ppm and TAN ranged from 0.00 – 1.59 ppm throughout the experiment. No graph was generated for pH or temperature because the values were virtually constant ($pH \approx 7.6 – 8.2$, temperature $\approx 21 - 24^\circ$ C) throughout the experiment. Similarly, the salinities of each tank were not graphed because they were constantly maintained throughout the experiment.

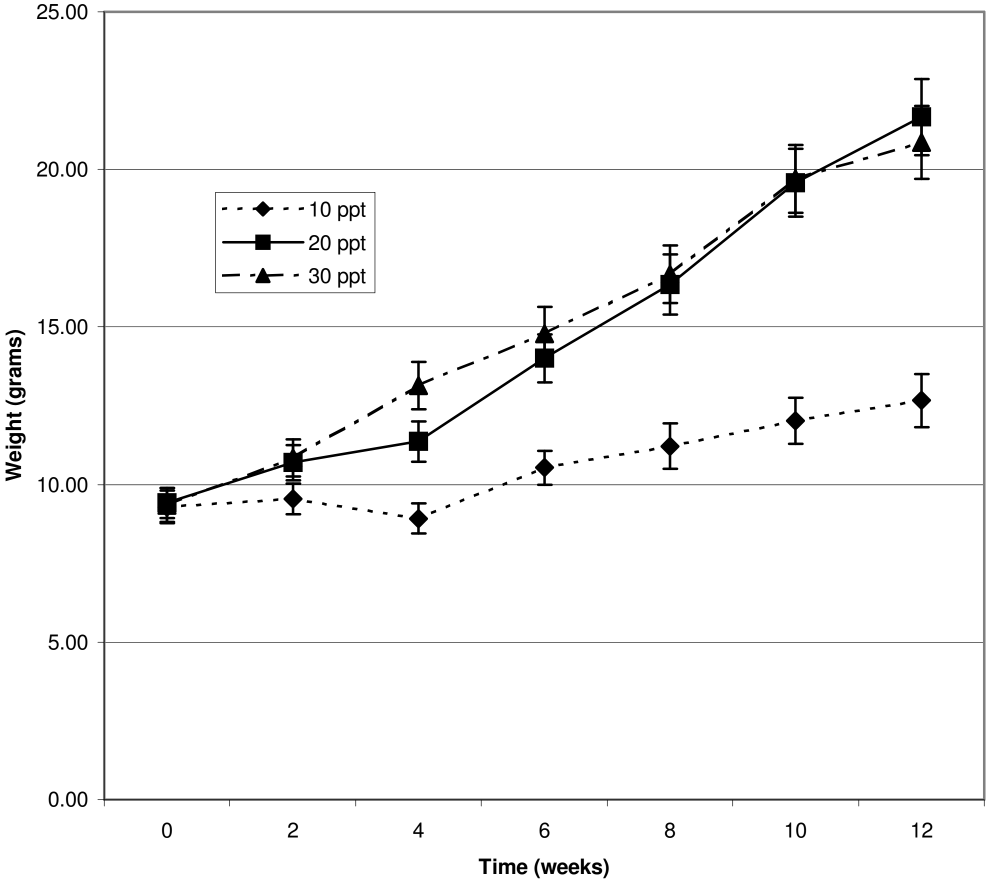

Figure 3.4 – Mean weight (± SE) versus time for juvenile black sea bass grown in closed tanks with 10 ppt, 20 ppt and 30 ppt salinities.

Table 3.2 – Initial and final weights, weight gain, relative growth rate (RGR), specific growth rate (SGR), daily weight gain (DWG), feed conversion ratio (FCR), and adjusted feed conversion ratio (FCR_{adj}) for juvenile black sea bass grown in closed tanks with 10 ppt, 20 ppt and 30 ppt salinities.

	Variable							
Salinity	Initial weight (g)	Final weight (g)	Weight gain (g)	RGR (%)	SGR (%/day)	DWG (g/day)	FCR	FCR_{adj}
10 ppt	9.29 ± 0.52	12.67 ± 0.84	3.38	36.4	0.38	0.042	– 8.84	8.09
20 ppt	9.42 ± 0.48	21.66 ± 1.20	12.24	129.9	1.03	0.151	2.99	2.37
30 ppt	9.35 ± 0.54	20.86 ± 1.15	11.51	123.1	0.99	0.142	2.86	2.46

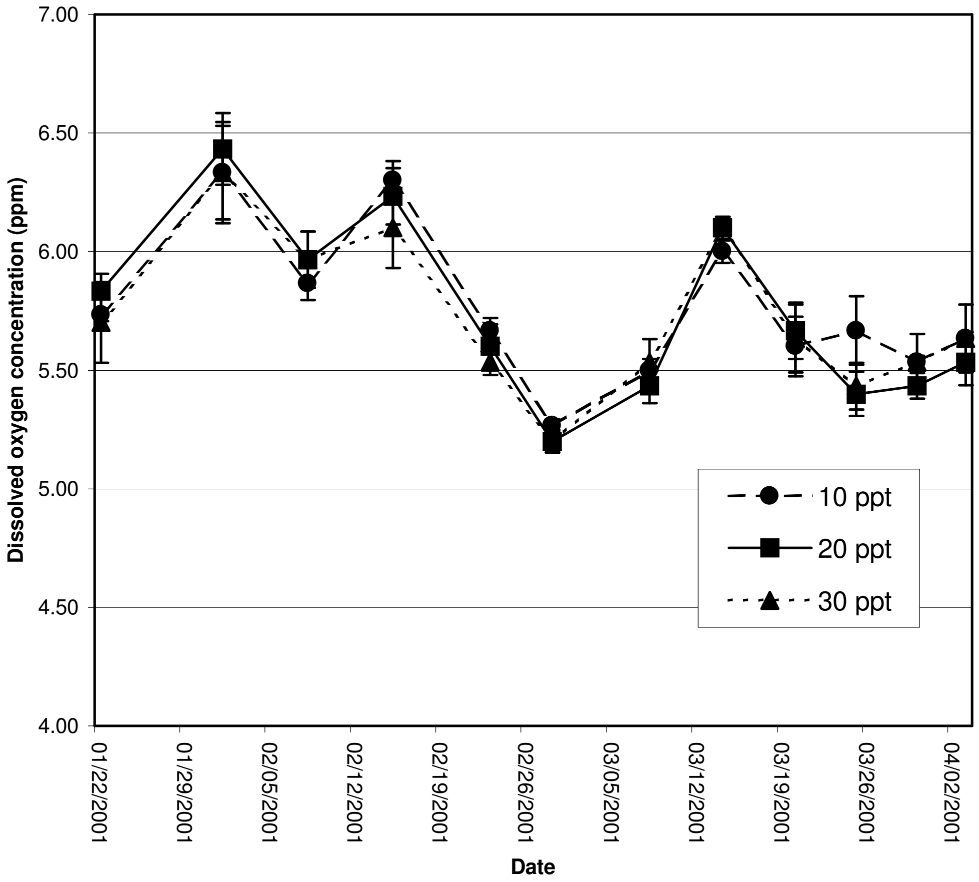

Figure 3.5 – Mean dissolved oxygen concentration (± SE) of each treatment for the duration of the salinity experiment, January 15 – April 6, 2001. Measurements were taken at least weekly and values of each replicate tank were averaged for each treatment.

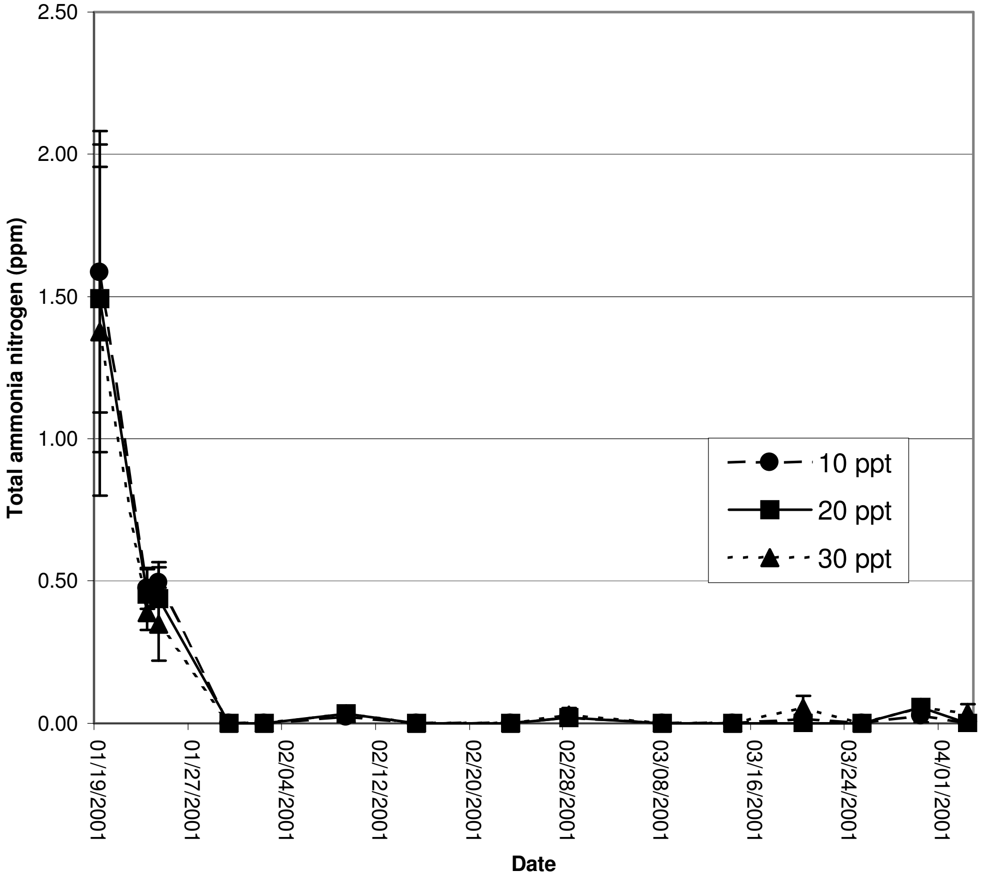

Figure 3.6 – Mean ammonia (TAN) concentration (± SE) of each treatment for the duration of the salinity experiment, January 15 – April 6, 2001. Measurements were taken at least weekly and values of each replicate tank were averaged for each treatment.

Discussion

Temperature

The results of the temperature experiment indicate that juvenile black sea bass grow optimally at 25º C. This is consistent with another temperature experiment with black sea bass. Berlinsky et al. (2000) found that larval black sea bass grew significantly larger at 22º C compared to 18º C. They also suggested that higher growth rates could be attained at higher temperatures. Similar temperatures have been reported for optimal growth of other subtropical and temperate fishes. McVey (1991) reports an optimal growth rate for European sea bass (*Dicentrarchus labrax*) at 22º C, with a tolerance range of 8º C to 27º C. Akatsu et al. (1983) report an optimal temperature of 30 – 31º C for growth of larval grouper (*Epinephelus tauvina*). Woiwode and Adelman (1991) report an optimal temperature range of 25.7 – 27.9º C for growth of juvenile hybrid striped bass (female *Morone saxatilis* x male *M. chrysops*), with a significant temperature-photoperiod interaction. Tomasso and Kempton (2000) report an optimal temperature of 30.4 – 31.1º C for red drum (*Sciaenops ocellatus*) fingerlings.

A previous temperature experiment designed identically to this experiment had to be abandoned because of a mycobacterial infection (*Mycobacteria marinum*). In this abandoned experiment, fish weight was 0.6 ± 0.1 grams at stocking. The infection manifested at 6 weeks and the experiment was terminated soon afterwards. However, at 2 weeks, significant differences in weight ($p < 0.0001$) were found between temperatures of 15º C, 20º C, 25º C and 30º C. In that experiment, Tukey's studentized range test ($\propto = 0.05$) found that fish grown at 30º C were significantly larger than fish grown in all other temperatures. Interestingly the abandoned experiment found the highest growth rate at 30º C, whereas this present experiment found the best growth rate at 25º C. One possible explanation for this apparent anomaly is that the brood stock that provided the juveniles for each experiment were from different populations. The abandoned experiment was performed with juveniles from a brood stock collected off the coast of Georgia, and the present experiment used juveniles spawned from a brood stock collected off the coast of Virginia. These differences were not known at the time of the experiments but rather were discovered in a later conversation with the commercial hatchery manager where the fingerlings were obtained. Mercer (1989) has indicated that black sea bass in the Atlantic Ocean are divided into two populations separated by Cape Hatteras, NC. The northern population would not experience temperatures as high as 30º C, whereas the southern population experiences this temperature annually.

Atwood et al. (2001) reported an upper thermal tolerance of 33.3º C and a lower thermal tolerance of 3.7º C. This explains the high mortality in the 30º C treatment since this temperature was near the upper thermal tolerance. The fish used in the Atwood et al. study were from the northern stock, as were the fish in this experiment.

Black sea bass can tolerate a wide range of temperatures in the wild. Cupka et al. (1973) report catching juvenile black sea bass from 5.6º C to 30.4º C in South Carolina. The majority of these fish were caught above 10º C, and the authors claim no temperature preference above 10º C. Similarly Musick and Mercer (1977) report catching fish in the Chesapeake Bight within a range of 6º C to 26º C with the majority being caught above 9º C. They also report that juvenile black sea bass were only caught in trawls in the York

River when bottom temperatures exceeded 9° C to 10° C. Mercer (1989) also reports black sea bass collections in temperature ranges of 6° C to 29° C in North Carolina.

These results will also aid in the understanding of the black sea bass population dynamics in the South Atlantic Bight. The southern population undergoes a major spawn in the spring and a minor spawn in the fall (Wenner et al. 1986). Those fish spawned in the spring will experience as much as eight months with water temperatures of 20° C or higher before winter arrives and retards their growth. Those fish spawned in the fall, however, will experience as little as one month with water temperatures of 20° C or higher. These fish, although of the same year class as the fish spawned in the spring, will presumably be smaller in size during their first years of growth.

Fish farmers need to understand growth rates at all of these temperature ranges if they intend to have outdoor tanks or tanks without any means of temperature regulation. The juveniles in this experiment lost weight in the 15° C treatment during the six-week experiment. This is unacceptable for a fish farmer, and he will need to utilize heaters during winter months as water temperatures in Georgia are typically lower than 15° C, and will be much colder in more northern latitudes. Fish farmers will also need to understand the degree of the difference in growth rate to determine how much heating will be required in the winter to balance optimal growth with the additional operating costs associated with increased water heating. Conversely, farmers will need to know how much chilling (if any) will be required in the summer to balance optimal growth with the increase in operating cost.

Salinity

The results of the salinity experiment indicate that juvenile black sea bass grow optimally at either 20 ppt or 30 ppt salinity. These results differ from another salinity experiment with black sea bass. Berlinsky et al. (2000) report a significant increase in growth of juvenile black sea bass at 20 ppt salinity compared to 32 or 10 ppt. The size of the fish was slightly different in the two experiments (3.7 g in the Berlinsky et al. experiment versus 9.4 g in this experiment) and could account for the conflicting results.

Atwood et al. (2001) showed that the lower threshold of salinity tolerance for black sea bass is 4 to 6 ppt. Berlinsky et al. (2000) also anecdotally mentioned 9 ppt as the lowest observable tolerance of salinity for black sea bass. The results presented in this experiment complement the findings of Atwood et al. (2001) and Berlinsky et al. (2000) by furthering our understanding of the effects of salinity on the growth of juvenile black sea bass.

Black sea bass can tolerate a wide range of salinity in the wild. Cupka et al. (1973) report catching juvenile fish from 8.8 to 37.8 ppt but most were caught above 30 ppt, with an overall increasing CPUE as salinity increases. Mercer (1989) also reports catching fish in salinities ranging from 1 to 36 ppt. Fish caught at the lower ranges of these reported salinities were presumably just passing through these hyposaline areas since Atwood et al. (2001) report a lower lethal threshold of between 4 and 6 ppt salinity. Another possibility is that the body of water in which the fish were caught was stratified. The authors might have sampled the hyposaline surface layer, while the black sea bass were residing in the higher salinity water of the bottom layer.

Repeating this experiment for sub-adult and adult black sea bass will be important. Cupka et al. (1973) and Musick and Mercer (1977) reported that juvenile black sea bass are known to inhabit estuarine areas as well as offshore reefs. Additionally, Waltz et al. (1979) showed a positive correlation between black sea bass age and distance offshore. This suggests that older black sea bass might not be able to tolerate a salinity range as low as that of the juveniles in this experiment. Therefore, older black sea bass may prove to exhibit significantly lower growth rates at 20 ppt when compared to 30 ppt salinity.

A similarly designed salinity experiment (10 ppt, 20 ppt, and 30 ppt) attempted for sub-adult black sea bass in 1200-L recirculating tanks had to be abandoned because of a bacterial infection (*Vibrio* sp.). Although data were not obtained from the experiment, initial observations indicated that sub-adult black sea bass (193.4 ± 14.4 g) can tolerate 10 ppt salinity for periods longer than one month. Additionally, the fish in the 10 ppt treatment exhibited very hearty appetites and activity levels. Interestingly, the fish in this lowest salinity treatment exhibited greater survival than the fish in the 20 ppt and 30 ppt treatments. Eventually all fish in the 30 ppt treatment and most of the fish in the 20 ppt treatment succumbed to the infection. Nearly all of the fish in the 10 ppt treatment survived the episode, perhaps resulting from an antiseptic effect of the lower salinity. After termination of the experiment, the surviving fish were kept in captivity for another year in flow-through tanks (ambient salinity of the Skidaway River) with no obvious long-term health problems.

Although growth was significantly reduced and mortality was significantly higher in the 10 ppt treatment of the present experiment, the fish exhibited positive growth at that salinity. For aquaculturists intending to grow black sea bass in facilities located some distance inland, the salinity of the available water source may be approximately 10 ppt. In addition, one farmer intends to make artificial seawater by using well water and "Instant Ocean" salt mix. Knowing the degree of difference in growth rate at 10 ppt, 20 ppt and 30 ppt will be important in determining acceptable losses in growth in terms of cost of increasing salinity with artificial salt. With the high mortality, high FCR and low growth rates exhibited in this experiment, raising juvenile black sea bass in water with 10 ppt salinity for extended periods of time would not be advisable. Rearing marine fish in lower salinities may eventually prove to be a useful tool for managing parasites, such as the dinoflagellate *Amyloodinium*, which has been problematic in red drum (*Sciaenops ocellatus*) aquaculture.

Water quality was never problematic during either experiment (Figures 3.2 – 3.3, 3.5 – 3.6). Average ammonia (TAN) concentrations were occasionally recorded at levels above 1 ppm in both experiments, and as high as 3.5 ppm in one tank of the salinity experiment, but these levels never seemed to adversely affect the fish. Additionally, Hoff (1970) noted that the fish in his experiments experienced dissolved oxygen levels as low as 3 ppm for periods of 1 – 3 days "with no apparent effect" on the fish.

Black sea bass have exhibited hardiness, excellent market potential, fast growth and tolerance of deleterious water quality (Hoff 1970; Roberts et al. 1976; Harpster et al. 1977; Tucker 1984; Kim 1987; Costa and Provenzano 1993; Berlinsky et al. 2000; Kupper et al. 2000; Walker and Moroney 2000). Black sea bass also quickly adapt to a tank environment and readily accept artificial food (Hoff 1970; Kim 1987; Berlinsky et al. 2000). With this high resilience, adaptability, and desirable market value, this fish is highly recommended for mariculture.

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CHAPTER 4

CONCLUSIONS

The methodology of spawning and hatchery care of black sea bass has been studied extensively (Hoff 1970; Roberts et al. 1976; Tucker 1984; Chappell et al. 2001; Stuart et al. 2001) but research is lacking in the optimal growth requirements of hatchery-reared fingerlings. The research presented here is very timely and will serve to complement the existing body of literature on black sea bass mariculture, particularly experiments involving juveniles (Harpster et al. 1977; Kim 1987; Berlinsky et al. 2000).

In these experiments, optimal feeding ration, commercial diet, temperature, and salinity were determined for growth of juvenile black sea bass. In addition, this work is part of an ongoing effort to determine the feasibility of growing hatchery-reared fingerlings to a desirable market size of approximately 900 grams. The long-term goal is to provide a method that is economically and energetically advantageous to the current method of trapping adult and sub-adult wild fish and raising them to market size.

As the industry continues to develop, aquaculturists will need information concerning optimal diet and feeding ration for black sea bass. This research shows that growth rate can be increased significantly by changing from a trout feed to a salmon feed. In this diet experiment, juvenile black sea bass were shown to grow best on a high protein commercial feed. Specifically, this experiment compared growth rates for fish fed four commercial diets, Rangen Trout and Salmon Starter, Nelson and Son's Silver Cup Trout

Crumbles, Nelson and Son's Silver Cup Salmon Crumbles, and Zeigler Salmon Starter. Highest growth rates were obtained by using the Zeigler feed, which has a 50% protein and 15% lipid composition. Additionally, a feeding regimen of 5% daily ration (dry weight of food/ wet weight of fish) will save money for the fish farmer as well as increase production for his facility. Feeding ration is a dynamic function and should be closely monitored so that feed is not wasted. Aside from the capital loss, feed wastage also contributes to deleterious water quality and will slow growth or possibly lead to fish kills if not quickly ameliorated.

Aquaculturists will also need information concerning the effects of temperature and salinity on growth rates of black sea bass. These results show a significant increase in growth of fish in water temperature of 25º C when compared to 15º C, 20º C, and 30º C. Additionally, a significant increase in growth rates was observed in salinities of 20 ppt and 30 ppt when compared to 10 ppt.

Proximity to estuarine water and geographic location of aquaculture facilities will play major roles in determining the temperature and salinity regimes that fish farmers experience in their respective locales. In fact, one potential fish farmer in coastal Georgia has proposed mixing synthetic salt with well water to supply his black sea bass mariculture facility. In addition, facilities in the northern United States will experience much colder winter temperatures than facilities in the South. Therefore, aquaculturists need to understand the effects of these parameters on the growth of their fish. Furthermore, they can use the data presented herein to develop operating budgets and determine the feasibility of optimizing growth parameters year-round at their facilities.

As existing aquaculture facilities endeavor to culture new species to diversify production, this type of research will benefit them greatly by presenting a formula for optimal growth of juvenile black sea bass. One should merely apply the optimal parameters recommended in these experiments (diet = Zeigler Salmon Starter; daily feeding ration = 5%; temperature = 25° C; salinity = 20 ppt or 30 ppt) to achieve desirable growth rates for black sea bass.

These results are applicable to juvenile black sea bass of approximately 1 – 20 grams in weight. More research is needed to fully understand how these factors change throughout the life cycle of the fish. Feeding ration, for instance, is a function of the size and age of fish and needs to be periodically adjusted to meet the changing metabolism of a growing fish. Younger fish grow at a faster rate than older fish and therefore require a higher daily food intake (as a percentage of their body weight) than older fish. Also, in the case of black sea bass, older fish tend to be strictly offshore whereas juveniles frequent estuarine areas. Waltz et al. (1979) found significant differences in age at depth with older, larger fish being found in deeper waters, farther offshore. Therefore, the results of the salinity experiment presented here may not apply to adult fish as they typically do not reside in estuarine areas with variable salinities.

Future investigations of possible interactions between diet, feeding ration, temperature, and salinity will be necessary to improve the understanding of how these parameters affect growth. Imsland et al. (2001) found an interaction between temperature and salinity on the growth of juvenile turbot (*Scophthalmus maximus*). Similarly, Boehlert and Yoklavich (1983) found a correlation between temperature and feeding ration for juvenile black rockfish (*Sebastes melanops*). Other interactions likely exist between these growth parameters and should be investigated for both juvenile and adult black sea bass. Future investigations can lead to the formulation of a growth model for culturing black sea bass. This model can be continually updated as other growth parameters are investigated in the future.

The results of these experiments indicate that these fish can be grown in a variety of environmental conditions and tolerate a wide range of water quality parameters. This conclusion is in agreement with the findings of previous black sea bass mariculture experiments (Hoff 1970; Roberts et al. 1976; Harpster et al. 1977; Tucker 1984; Kim 1987; Costa and Provenzano 1993; Berlinsky et al. 2000; Kupper et al. 2000; Walker and Moroney 2000; Copeland et al. 2001). Among the comments of these authors regarding the advantages of black sea bass mariculture are the ease of tank adaptation, ready acceptance of commercial fish feed, tolerance of a variety of water quality parameters, hardiness, and rapid growth rate. With such favorable characteristics and a superb market value, black sea bass appears to be an excellent candidate for mariculture.

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